the normal range, a decrease in the red cell count and megaloblastic or erythroblastic hyperplasia of the bone marrow. Human pernicious anemia and sprue respond to the parenteral administration of liver extract, but are not benefited by any substance of known chemical constitution that has been tested. The criteria for an experimental anemia analogous to pernicious anemia and sprue may therefore be given as: (1) Anemia of the macrocytic hyperchromic type; (2) hyperplasia of the erythroblastic tissue of the bone marrow; (3) response to purified liver extract given parenterally; (4) no response to other factors known to be essential in normal nutrition and metabolism.

Fifteen bile fistula dogs have developed anemia with increases in MCV and MCHb. No dog that has survived the operation for 4 months has failed to develop these blood changes. The highest MCV observed is 98.7; 11 of the 15 dogs have shown MCVs above 88.9 (4 S.D. above the mean for normals). The MCV in every dog has remained above 84.5 (3 S.D. above the mean) for considerable periods. Changes in MCHb have not been as striking as those in MCV. They have exceeded 25.7 (3 S.D. above the mean for normal animals) in 8 dogs and have been above 24.1 in the other 7. In 6 animals the MCD has been determined with a filar micrometer during the height of the anemia and has been found to exceed 3 S.D. of the mean for normal animals in each instance, the maximum diameter attained being 7.4 micra.

This anemia usually appears within 2 to 4 months after operation, shows (like pernicious anemia in man) a tendency to spontaneous remissions with reticulocyte showers, and is accompanied (as in sprue) by marked weight loss.

We are indebted to Dr. L. W. Diggs for reports on the condition of the bone marrow, the specimens being obtained by autopsy or biopsy. He informs us that the marrow exhibits a mild erythroblastic hyperplasia, and that the mean diameter of the nucleated red cells decreases following liver therapy.

The response to liver extract (Eli Lilly and Company, purified) given intramuscularly has been studied in 6 animals. In every instance a single injection of 30 units or more has been followed by a reticulocyte increase (2 to 20 per cent.), and a return of MCV and MCHb to within 2 S.D. from the normal mean. Increases of a million or more cells have been produced, but have required frequent injections totaling more than 30 units.

In order to prevent deficiencies that occur when bile is absent from the gastrointestinal tract it has been necessary to inject vitamins A, D, E and K. If these substances are not given parenterally the dogs are likely to succumb when the red cell count falls below 4.0 millions. In addition we have given various animals injections of thiamin, riboflavin, nicotinic acid, pyridoxin, calcium pantothenate and choline without improvement in the blood picture.

Incidental observations are the presence of acid in the gastric secretion, the absence of plasma bilirubin (which in any case is not detectable in the plasma of normal dogs), the presence of normal concentration of plasma protein and a susceptibility to the development of localized infections at the site of subcutaneous injections.

The anemia in the bile fistula dog fulfils the criteria that we have established for an experimentally produced APA factor deficiency analogous to pernicious anemia and sprue. We believe that this deficiency is probably due to a failure of absorption of the APA factor from the intestine. One must consider that the lack of recirculation of bile might interfere with the functions of the liver, and therefore produce a macrocytic anemia that could best be compared to the macrocytic anemia of human liver disease. But this latter condition usually fails to respond to liver therapy and in this respect differs from pernicious anemia, sprue and the anemia of our animals.

It is evident that a readily available experimental macrocytic anemia responsive to liver extract may make available a method of assay for this extract, which must now be standardized on pernicious anemia patients. We hope that continued study of this macrocytic anemia will also help to clarify the etiology of the macrocytic anemias of man.

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DIFFERENTIAL INHIBITION OF PHOTOCHEMICAL AND DARK REACTIONS IN PHOTOSYNTHESIS BY INORGANIC COMPOUNDS

IT has been known for a number of years that certain organic compounds, notably urethanes and cyanides, are capable of inhibiting one or the other of the reactions in photosynthesis. Studies recently completed have shown that various inorganic compounds can likewise inhibit the light and dark reactions separately. Cells of *Chlorella vulgaris* were suspended in test solutions for 20 minutes. They were then rinsed with water and their rates of photosynthesis were determined over a wide range of light intensities by means of Warburg manometers.

The results are shown diagrammatically in Fig. 1, in which the rate of photosynthesis is plotted as a function of light intensity on logarithmic scales. The control or normal curve is used as the basis of com-

parison, and the general types of curves obtained for treated cells are illustrated.

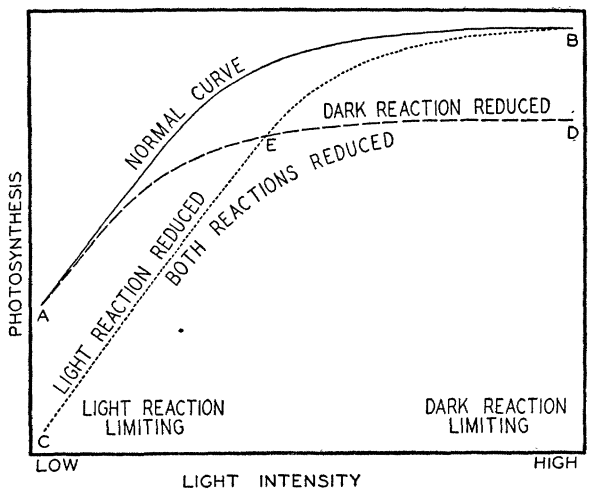

FIG. 1

The curves are interpreted according to the limiting-factor hypothesis of Blackman.[1] Thus, in the normal curve AB, the rate of photosynthesis rises with increase in light intensity over the low intensity range; here light limits or determines the rate of the whole process of photosynthesis. But at higher light intensities the curve flattens, since here light is in excess and some factor controlling the dark reaction limits the rate. In the same sense, at low light intensities the photochemical reaction is limiting and at high light intensities the dark reaction is limiting.

Treatment of cells with $ZnSO_4$, $NiSO_4$ or KCl resulted in a curve of the type AED. These substances retard only the dark reactions of photosynthesis, since their depressing effect is negligible at low light intensities, and is greatest at high light intensities, where the photochemical reaction is in excess and the dark reaction determines the rate of the whole process. In their ability to reduce the dark reaction, these salts resemble hydrocyanic acid[2] and heavy water.[3] It is known that low temperature or deficiency of carbon dioxide gives a similar curve and that cells cultivated in weak light respond in the same manner.

If a substance affected the photochemical reaction alone, greatest inhibition of photosynthesis, as shown by greatest divergence of curves for control and treated cells (as between A and C of curves AB and CEB), would be evident at low light intensity where the photochemical reaction determined the photosynthetic rate, and retardation would become negligible at the highest light intensity. None of the inorganic substances tested gave a curve like CEB, but phenylurethane in Warburg's[2] experiments approximated this type. Chlorophyll deficiency has a similar effect.

Treatment with $CuSO_4$, H_3BO_3, KI, $CoSO_4$ and $(NH_4)_2SO_4$ inhibited photosynthesis over a wide range of light intensities and resulted in curves which were variations of the composite curve CED. It is evident that these substances inhibited both the photochemical and the dark chemical reactions.

Copper sulfate, known to be highly toxic to algae, was found to depress photosynthesis in *Chlorella* exposed for 20 minutes to a 10^{-7} molar solution. In contrast, treatment with 0.4 M $MnSO_4$ had no apparent effect, and *Chlorella* withstood equally high concentrations of KNO_3 and $MgSO_4$. Solutions of higher osmotic pressure, however, depressed photosynthesis and tests with hypertonic sucrose solutions demonstrated that this effect was an inhibition of the dark reaction.

Since inorganic compounds are capable of differential inhibition of the reactions in photosynthesis, their use may provide new evidence regarding the mechanism of the process.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

AN IMPROVED METHOD FOR THE CONTINUOUS MEASUREMENT OF THE RATE OF OXYGEN CONSUMPTION FOR HUMAN SUBJECTS[1]

THE purpose of the following note is to describe a simple method for the conversion of an ordinary clinical basal metabolism machine to one where continuous measurement of oxygen volume consumption for a subject whether at work or at rest is possible. The accompanying figure shows schematically how this is accomplished. Considering first the apparatus to the left of the dotted line down the middle, one recognizes the usual components of the closed-circuit clinical machine, namely, the mouthpiece (A), triple valve (B), respiration spirometer (C), soda-lime bottle (D) and motor-blower (E). A heat exchanger (F) may be included in the circuit to cool the respiration system. The modification to be described could apply just as well to the clinical machine with soda-lime container and flutter valves inside the spirometer instead of an outside soda-lime bottle and motor-blower.

In our modified apparatus, as the subject metabo-

[1] F. F. Blackman, *Ann. Bot.*, 19: 281–295, 1905.

[1] Contribution No. 32 from the John B. Pierce Laboratory of Hygiene, 290 Congress Avenue, New Haven, Connecticut.

[2] O. Warburg, *Biochem. Zeitschr.*, 100: 230–270, 1919.

[3] F. N. Craig and S. F. Trelease, *Amer. Jour. Bot.*, 24: 232–242, 1937.