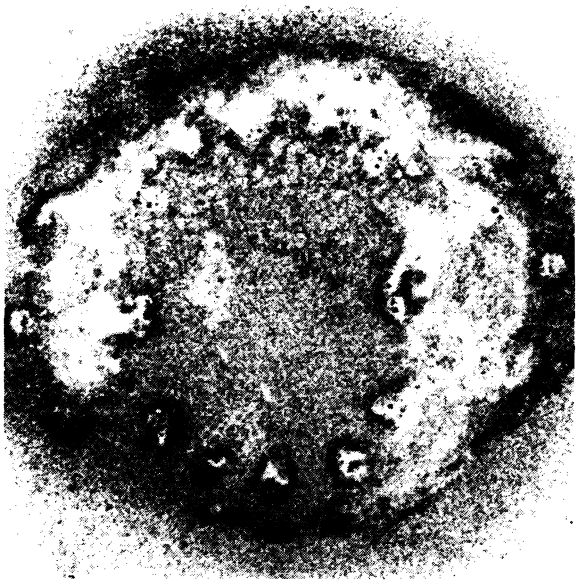

FIG. 3. Radioautograph of section of alligator weed at a treated node. (Dark spots indicate accumulation of 2,4-D-C^{14}.)

Radioautographs were made of sections in various positions of the plant and with various types of film. Eastman contrast lantern slide plates and Ansco stripping film were too slow and required long exposures. Ansco nonscreen x-ray film worked well, with exposure flux of 6 million β-particles/cm². This is six times the flux reported by Calvin (*3*). The films were developed in Kodak D-19. Individual nodal or internodal sections did not give sufficient activity to secure a suitable radioautograph when the 2,4-D-C^{14} was applied at only one point on the plant. If adjacent pairs of leaves were treated and a section taken between, good pictures were obtained.

The normal methods of preparing histological sections by dehydration and paraffin embedding would remove most of the 2,4-D-C^{14} in the section. Since the energy of the emitted β-particle is low, thick sections cut manually with a razor blade could be used, for the only effective radiation will be from the tissue layer nearest the film. The radiation from the more distant portions of tissues would be absorbed. In the preliminary experiments the stem sections dried out readily and distorted markedly, not producing satisfactory radioautographs. Interposing thin films between the x-ray film and the section did not prove satisfactory. The problem of distortion was solved by making the radioautographs in a plastic box with a piece of filter paper lining the bottom of the box and a small film support inside. If the filter paper was kept moist, the tissue sections were kept from dehydrating and distorting. Lead foil strips were placed between the films to cut down background fogging from adjacent sections. The plastic box was made light-tight by sealing with masking tape and, as an additional precaution, was placed inside a sealed box. A typical radioautograph is shown in Fig. 3.

Additional radioautographs must be taken before a complete interpretation may be made. The botanical significance of these results will be reported later.

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Purification of the Anticoagulant Principle Obtained from the Indian Cattle Leech, *Hirudinaria*

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Lal and Chowdhury (*1*) pointed out that the anticoagulant substance obtained from the Indian cattle leech, *Hirudinaria,* was not only nontoxic but quite effective *in vitro* and also *in vivo* in white mice, both intravenously and orally. In their experiments a crude extract of the anticoagulant substance was obtained by simple maceration of the leech heads in distilled water, and separation of proteins was effected by coagulation at 80°–85° C in a water bath for 20–30 min. The protein content of the anticoagulant substance at this stage was found to be 600 mg%. In our attempts to obtain the active anticoagulant principle in purer form several methods were tried.

To the crude extract obtained after maceration of the leech heads in distilled water was added trichloracetic acid in sufficient quantity to make a 4% solution of the acid for the precipitation of the proteins. The mixture was kept overnight and was subjected to filtration and centrifuging at 2,500 rpm the next day. The supernatant fluid was buffered with phosphate buffer and pH adjusted to 7.2, but a large quantity of phosphate buffer had to be used in order to adjust the pH. The fluid was then evaporated to dryness on a water bath and left in a desiccator overnight. The residue when dissolved in isotonic saline was found to exhibit the anticoagulant activity. The protein content at this stage was estimated and found to be 224 mg%. As the quantity of phosphate required to adjust the pH is very large, the method does not seem satisfactory for obtaining the active substance in pure form.

In another method the macerated extract of leech heads in distilled water was kept at 80°–85° C for 20–30 min and filtered. Absolute alcohol in sufficient quantity was added to the filtrate to precipitate the proteins, and the mixture was left overnight. Next morning it was filtered and then centrifuged to obtain a clear supernatant fluid, which was evaporated to dryness on a water bath to remove the alcohol. The residue was once more dissolved in distilled water, and the solution so obtained was again evaporated to dryness on a water bath to remove the remaining traces

of alcohol. The residue thus obtained when dissolved in isotonic saline showed a total loss of the anticoagulant activity. The protein content of the residue at this stage was found to be about 48 mg%.

Simple dissolution of the crude extract of the leech heads in distilled water, separation of proteins by coagulation at 80°–85° C for 20–30 min, filtering and evaporating to dryness on a water bath, and leaving in a desiccator overnight gave very satisfactory results. Usually, after 8–10 such treatments, the residue obtained was easily soluble in water, yielding a clear solution. An isotonic saline solution of the residue showed an effective anticoagulant activity. The protein content of the anticoagulant at this stage was found to be about 90–100 mg%.

In yet another case the crude extract, after treatment at 80°–85° C for 20–30 min, was mixed with isotonic saline and left overnight. Next morning it was filtered; the solution showed an active anticoagulant property, and the protein content was found to be approximately 80 mg%. This method seems capricious, however, as it gave variable results.

Attempts to obtain a completely protein-free substance were not successful. Even when the biuret test was negative, the estimations done by a biophotocolorimeter using Greenberg's phenol reagent method indicated the presence of small traces of protein. Glass-distilled water (pH 7–7.2) was used in the above experiments, which were performed at room temperature (100°–108° F), and thymol was used as a preservative. Further work on the biochemical estimations of the anticoagulant is in progress and will be published later.

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Book Reviews

Time's Arrow and Evolution. Harold F. Blum. Princeton, N. J.: Princeton Univ. Press, 1951. 222 pp. $4.00.

"To what extent has the course of evolution been determined by strictly physical factors that have permitted no exercise of natural selection and to what extent have the former set limits within which the latter may act?"

"How, when no life existed, did substances come into being which today are absolutely essential to living systems, yet which can only be formed by those systems?"

Most readers will agree that Dr. Blum has made a profound contribution in his formulation of these questions and in his presentation of the facts bearing upon their answers. The problem is like a great jigsaw puzzle in which small areas of the picture have been put together but whose final solution will require many trials and failures before the correct combinations are discovered. The book will cause vigorous discussion, and its virtues lie in the areas where the author has offered clarification of these broad questions.

Blum suggests that "time's arrow," the second law of thermodynamics, is the unyielding warp upon which the great tapestry of evolution has been woven. Many experts in the field of thermodynamics will not wish to apply the second law to a single photon, in the form of an x-ray, acting upon a gene to alter the process of evolution. Irving Langmuir has called this a divergent phenomenon and concludes "In a world in which divergent or quantum phenomena occur we can have no absolute relation of cause and effect." However, the author is certainly correct in emphasizing the restrictions that chemistry and possible chemical changes have placed upon the course of evolution.

The origin of the compounds in the first living cell is, indeed, a problem of singular difficulty. In a cosmic cloud, with its large excess of hydrogen, the compounds in living matter are generally unstable with respect to methane, water, and ammonia. The earth's early atmosphere was doubtless water, carbon dioxide, and some ammonias. High energy photons acting upon these compounds certainly produced many free radicals, and the combination of these radicals gradually led to more and more complicated molecules. The author assumes that compounds as complicated as adenylic acid were formed in the first billion years. This conclusion also will be a point of controversy, but the existence of living matter surely gives weight to his contentions.

Time's Arrow and Evolution will interest readers from all fields of science and a discussion of its propositions should broaden the attack upon these fundamental problems.

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Metallurgical Thermochemistry. O. Kubaschewski and E. Ll. Evans. New York: Academic Press; London: Butterworth-Springer, 1951. 368 pp. $6.00.

This book presents an extensive collection of thermodynamic data, an introduction to their use in metallurgical calculations, and descriptions of experimental procedures by which they are determined. In explaining the title the authors state that