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Manuscript received April 14. 1952.

Separation and Detection of the Pyrethrin-Type Insecticides and their Derivatives by Reversed Phase Paper Chromatography[1]

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For the purpose of studying the metabolic fate of C^{14}-labeled insecticides of the pyrethrin type a method was required for the separation of these esters and their acid and alcohol products of hydrolysis. It was necessary to separate these materials, under conditions unfavorable to their further decomposition after extraction from insect tissue, etc., on unidimensional paper chromatograms so that they could be assayed radiometrically by the scanning techniques described elsewhere (*1*). The method of reversed phase paper chromatography developed for the separation of the bromine analogs of DDT and its derivatives (*2*) has been found to be applicable, with slight modification as follows:

Whatman No. 1 paper in 1-in. strips is washed by soaking for 30 min in a mixture consisting by volume of 45% ethanol, 50% water, and 5% conc HCl. The strips are then successively soaked in dilute aqueous ammonia and distilled water, and finally drained and dried. For the reversed phase paper chromatography of the esters the washed strips are impregnated with petroleum jelly (USP) by dipping once in a 3% (w/v) solution of petroleum jelly in diethyl ether, draining, and drying. An ethereal solution containing not more than 100 μg of the mixture to be resolved is applied near the bottom of a strip, and the solvent, consisting by volume of 45% ethanol, 50% water, and 5% aqueous ammonia (sp gr, 0.90) allowed to ascend the strip in the usual way in an atmosphere of nitrogen saturated with the solvent vapor. After 24 hr the strips are dried, sprayed with 0.1% neutral aqueous potassium permanganate, immediately rinsed with distilled water until quite free of permanganate, and partially dried. While still damp the strips are sprayed with 0.5% benzidine in dilute aqueous acetic acid (*3*) The MnO_2 formed in the presence of unsaturated compounds of the pyrethrin type appears as intense blue zones, less than 1 μg of the pyrethrins or their hydrolysis products being easily detected.

Allethrin, the allyl homolog of cinerin I, runs with an R_f value of 0.40, whereas the allyl cinerolone and chrysanthemum monocarboxylic acid products of hydrolysis run together with an R_f value of 0.89. When a concentrate of natural pyrethrins, labeled as containing 43% of "pyrethrin I" and 37% of "pyrethrin II" was resolved by this method, two major constituents running with R_f values of 0.23 and 0.72, respectively, and at least three other constituents running with R_f values of 0, 0.12, and 0.90 were detected. The composition of the mixture in terms of the true pyrethrins and cinerins I and II was unknown. The two major zones were believed to be the so-called pyrethrins I and II, respectively, since pyrethrin II is known to have a partition coefficient more favorable to the polar or mobile solvent phase. Evidence in support of this interpretation was obtained in two ways. First, when the zones separated on a second strip were sprayed with 0.05 *N*-ethanolic KOH containing 0.02% thymolphthalein, the faster running major zone required considerably more spraying than the slower running zone before the permanent blue ($pH > 10$) was obtained. This indicated the higher saponification value which would be expected of the dicarboxylic acid esters of "pyrethrin II." Second, a third strip was cut into 1-in. sections, which were rinsed separately in 2 ml petroleum ether containing 2 μl light mineral oil. The rinse of each section was transferred to a 2×4 cm exposure vial and bio-assayed with adult female houseflies by the methods described by Hoskins *et al.* (*4, 5*). Insecticidal activity, as shown by "knockdown" and mortality after 24 hr, was exhibited only by the substances running with R_f values of 0.23 and 0.72, and, qualitatively, the slower running component was the more toxic to houseflies. This is in agreement with Gersdorff (*6*), who found the monocarboxylic acid esters to be the more toxic. Some unsaturated $KMnO_4$-reactive material is present in the neutral ether or acetone extracts of macerated housefly tissue but remains at the point of application in the reversed phase chromatograms and does not, therefore, interfere with the detection of the extracted insecticides.

The alcohol and acid products of allethrin hydrolysis which run together on the reversed phase paper chromatogram may be separated as follows: The mixture is applied near the bottom of a washed strip that has not been impregnated with petroleum jelly. Petroleum ether (boiling range, 35°–60°), saturated with 10% aqueous HCl, is allowed to ascend the strip for about 10 min (atmosphere saturated with the solvent). The strip is dried and then rechromatographed with light

[1] The gifts of samples of allethrin and their derivatives by S. H. Harper and J. B. Moore are gratefully acknowledged.
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petroleum ether saturated with aqueous ammonia (sp gr, 0.90) the solvent being allowed to ascend for about 30 min. The strips are finally dried and sprayed with the permanganate and benzidine reagents as above. Under these conditions, the allyl cinerolone barely moves from the point of application. The cis- or trans-chrysanthemum monocarboxylic acids remain at the final position of the acid solvent front as the ammonium salts, and the unchanged esters move with the ammoniacal solvent front. The application of these methods to the study of the metabolic fate of the C^{14}-labeled insecticides will be the subject of a later report.

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Manuscript received April 18, 1952.

Algae (*Chlorella*) as a Source of Nutrients for the Chick[1]

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Following the work of Spoehr and Milner (*1*) on the effect of environmental conditions on the chemical composition of *Chlorella pyrenoidosa,* interest has been shown concerning the possibility of using mass cultures of this green unicellular plant for the production of food. Myers *et al.* (*2*) studied the design of a growth chamber necessary for maximum growth rates of Chlorella. These papers have recently been reviewed (*3*). Nevertheless, little or no experimental work has been done involving the use of *C. pyrenoidosa* in animal feeding trials. Consequently, the value of dried Chlorella as a nutrient source has been tested with chicks.

Uniform groups of 16 day-old New Hampshire chicks of both sexes were maintained in electrically heated battery brooders with raised wire floors throughout a 4-week experimental period. Feed and water were supplied *ad lib.* Mortality, feed consumption, and body weights were recorded. The basal diet used in this study consisted of ground yellow corn, 61.7%; soybean oil meal (solvent), 34%; ground limestone, 1%; steamed bone meal, 3%; iodized salt, 0.3%; manganese sulfate, 0.025%; D-activated animal sterols, 0.05%; and 0.2 mg 2-methyl-1,4-naphthoquinone/lb. This ration is considered suboptimal in its content of riboflavin, vitamin B_{12}, and vitamin A activity for supporting rapid early growth of chicks. The Chlorella[3] had been dried *in vacuo* at a temperature of 70° F. When Chlorella was fed, it was substituted in the diet for an equal weight of soybean oil meal.

An amino acid assay of the dried Chlorella used in these feeding trials is given in Table 1, and a vitamin assay is given in Table 2. The sample had been grown under pilot plant conditions. It is reasonably similar in nutritive value to samples grown elsewhere under laboratory conditions, assays of which are also given in Tables 1 and 2. Aside from the low level of methionine, Chlorella protein compares favorably with soybean oil meal protein. The vitamin levels are relatively high as compared with many other important foods and feedstuffs.

TABLE 1

AMINO ACID ASSAY OF DRIED CHLORELLA

Nutrient	Pilot plant* sample (%)	Laboratory† sample (%)
Crude protein	44.0	40.0
Arginine	2.06	2.39
Histidine	0.62	0.65
Isoleucine	1.75	1.69
Leucine	3.79	1.99
Lysine	2.06	2.43
Methionine	0.36	0.57
Phenylalanine	1.81	2.14
Threonine	2.12	1.91
Tryptophane	0.80	0.41
Valine	2.47	2.67
Glycine	—	2.20

* Microbiological assay by Food Research Laboratories, Inc., reported to Carnegie Institution of Washington by Kenneth Morganridge, chief chemist, March 27, 1952.

† Mean values of two samples grown by Spoehr and Milner (*1*); microbiological assay by Merck & Co., 1949.

The various supplements added to the basal diet, together with the results obtained, are shown in Table 3. The inclusion of 10% Chlorella to the basal diet in place of an equal amount of soybean meal resulted in a very marked increase in growth and improvement in feed efficiency. This improvement is attributed primarily to the high riboflavin and carotene content of the Chlorella, although important quantities of several other B-complex vitamins are also supplied by this level of Chlorella. The addition of 0.1% DL-methionine (group 3) to the Chlorella-containing diet resulted in some improvement. However, the further addition of vitamin A, vitamin B_{12}, riboflavin, niacin, pantothenic

[1] Scientific Paper No. A369, Contribution No. 2362, of the Maryland Agricultural Experiment Station (Department of Poultry Husbandry).

[2] The author gratefully acknowledges the technical assistance of Hugh T. Lathroum in conducting this study. Appreciation is also expressed to John S. Burlew, Carnegie Institution of Washington, Washington, D. C., for supplying the Chlorella, and to Merck & Co., Rahway, N. J., for supplying the crystalline vitamins used in this investigation and for nutritional analyses of samples of Chlorella.

[3] The dried *Chlorella pyrenoidosa* (Emerson strain) was supplied by A. W. Fisher, Jr., of Arthur D. Little, Inc., Cambridge, Mass. It had been grown in their Chlorella pilot plant for the Carnegie Institution of Washington. The conditions of growth are described in *The Large-scale Culture of Algae,* a monograph edited by John S. Burlew, Carnegie Institution of Washington, Pub. No. 598 (1952).