excretory system arises as an outgrowth from the primary excretory bladder, the branches of which secondarily anastomose to form the network of vessels and spaces characteristic of the adults of the superfamily Strigeoidea. All the organs of the reproductive system are completely formed in the metacercaria but in a less mature state than in the adult.

Alaria mustelae, sp. nov.: characters of the genus. Length 0.82–1.74 mm, foliaceous forebody one to two times length of the cylindrical hindbody, entirely covered with retrorse spines. Oral sucker terminal, average width and length 0.082 x 0.082 mm, slightly larger than acetabulum, but shorter than pharynx, which averages 0.106 x 0.078 mm; ovary anterior to testes, averaging 0.152 mm in width; Mehlis' gland postero-lateral to anterior testis; uterine eggs few, averaging 0.11 x 0.072 mm. Cercaria fork-tailed, pharyngeate, flame cell pattern $2[(1+1+1)+(1+1+(2))]$, developing in slender unbranched sporocysts in the snail, *Planorbula armigera.* Agamodistomum stage in frogs and tadpoles. Metacercariae of diplostomulum type, in muscles and lungs of small frog-eating mammals.

Poche, 1925, split up the family Strigeidae Railliet, separating off the family *Cyathocotylidae* with *Cyathocotyle* Mühling as the type genus. It is here proposed to restrict the family Strigeidae still further by removing all those forms not belonging within the subfamily *Strigeinae* Railliet. The genus *Brauanina* Heider becomes the type of the family *Brauninidae* fam. nov., which is characterized by the great reduction of the hindbody, by the inclusion within the holdfast organ of all reproductive organs except terminal portions of genital ducts, and the possession of cirrus and cirrus-pouch. The genus *Alaria* Schrank is the type of the family *Alariidae* Tubangui, 1922, which includes the subfamilies *Alariinae* Hall and Wigdor, and *Polycotylinae* Monticelli. The genus *Cleistogamia* Faust is removed from the superfamily *Strigeoidea* since it does not have a holdfast organ, and possesses several characters not found within the superfamily *Strigeoidea.* These characters are those structures which make the cleistogamous type of fertilization imperative and the nonoperculate egg with filamentous appendages. The genus Cleistogamia Faust is placed in a new family *Cleistogamiidae* with the single subfamily *Cleistogamiinae* Faust, 1927. No suggestion is offered regarding its relationship to other families.

A more complete description of this parasite, its life history, and a discussion of the taxonomy of the Strigeoidea will appear elsewhere.

NELLY J. BOSMA

ZOOLOGY DEPARTMENT,
UNIVERSITY OF MICHIGAN

THE RELATION BETWEEN IRON, HUMIC ACID AND ORGANIC MATTER IN THE NUTRITION AND STIMULATION OF PLANT GROWTH

THIS communication will report briefly an extensive investigation just concluded in which the chemical substance contained in natural soil humic acid, chiefly responsible for the marked stimulation of growth of various higher and lower plants, has been determined. The growth studies have been conducted with the free-living aerobic soil bacterium *Azotobacter vinelandii,* and, although the findings apply strictly to this organism only, there are numerous reasons for believing them to apply quite generally to other organisms, in qualitative respects, at least. A wide variety of experimental conditions have been employed with respect to method of growth, criteria of growth and stimulation, nature of medium, source and concentration of nitrogen, temperature, pH, duration of experiment and humic acid. The results are being described in detail elsewhere,[1] but in view of their general implications and interest are being summarized here.

Both natural soil humic acids and synthetic humic acids have been employed. The former were prepared by washing soil with N HCl and then extracting with 10 per cent. KOH, the latter by boiling 20 per cent. glucose in 35 per cent. HCl and then centrifuging and extracting with 10 per cent. KOH. Purification was accomplished in both cases by precipitating at pH 3, centrifuging, and redissolving at pH 7.5, the cycle being repeated five times in all. It was possible to prepare, by minor variations in method and soil used, natural humic acids varying in iron content from 0.06 to 1.4 per cent. By adding $Fe_2(SO_4)_3$ to the glucose-HCl mixture during boiling, it was possible to prepare synthetic humic acids varying in iron content from 0.03 to 4.4 per cent.; other metals could be introduced similarly into the synthetic humic acids.

It has been determined that natural humic acid increases growth primarily, if not entirely, by virtue of the *iron* it contains; the organic fraction is substantially inactive, and likewise other inorganic impurities. The following evidence supports this finding.

(a) Natural humic acid may be substituted more

[1] "The Chemical Nature of Humic Acid Growth Stimulation in Relation to Iron" and "The Physiological Nature of Humic Acid Stimulation of *Azotobacter* Growth," submitted for publication to *Soil Science.* These papers contain some seventy-five tables and figures of experimental data and represent several thousand experimental cultures. The relation of the present investigation to previous similar ones will not be discussed here, beyond mentioning the following recent pertinent papers: Clark, SCIENCE, 71, 269 (1930); Hopkins, *Bot. Gaz.,* 89, 209 (1930); Olsen, Compt. rend. Lab. Carlsberg, 18, 1 (1930); Iwasaki, *Bioch. Zeit.,* 226, 32 (1930); Blumenberg and Blumenberg, U. S. Patent 1,783,694, Dec. 2 (1930); Farries and Bell, *Ann. Bot.,* 44, 424 (1930); and Ashby, *Ann. Bot.,* 43, 805 (1929).

or less satisfactorily by complex non-ionized iron combined with an organic acid, such as ferric citrate, tartrate, oxalate; by inorganic ionic iron such as ferric sulfate, metallic iron; and by synthetic humic acids containing considerable percentages of iron. In any given experiment there may be a certain variability in response to the four different iron types, depending upon the particular nature of the experiment, and inevitable secondary differences between the types. A statistical study of the mass of data available shows, however, that *a given amount of soluble iron gives the same order of stimulation independently of whether the iron is added as in natural humic acid or as in any of its substitution compounds.* This statement is made with the provision that the experiments be allowed to proceed long enough to permit complete physiological consumption of the iron added, so as to avoid any limitation through induction period differences as described below. Iron stimulation may be observed often upon addition of as little as 0.01 p.p.m. Fe. The minimum concentration yielding approximately optimum stimulation, however, is about 0.5 p.p.m., under conditions where significant decreases in the iron concentrations being studied do not occur during the period of measurement. As the iron is later consumed in growth, as a function of time, the minimum optimum concentration of initially added iron may shift to as high as 5 to 10 to 15 p.p.m., causing 5 to 20-fold increases in growth. As a corollary to these quantitative relations, it may be stated that, as the percentage of iron contained in a compound increases, so stimulation increases, for any given concentration of natural or synthetic humic acid, or organic acid, iron compound. In considering inorganic iron it is important to observe that, although the last three types of compounds are completely soluble, inorganic iron is soluble in the majority of neutral media to the extent of only 0.01 to 0.05 p.p.m., which is considerably below the minimum optimum concentration of about 0.5 p.p.m. Owing to this solubility limitation inorganic iron will often yield, especially in experiments of relatively short duration, less satisfactory stimulation than the other three types, when comparison is made upon the basis, not of soluble iron, but of total iron added to the medium.

(b) Natural humic acid was not substituted by humic acids prepared synthetically from glucose to contain no more than traces of iron; by iron-free synthetic humic acids containing various metals such as Al, Co, Mn, Si, Mo and P; or by salts of such elements as Al, Co, Mn, Mo, Ni, Zn, Cr, Cu and Ag, at various concentrations ranging from 0.01 to 50 p.p.m.

(c) Both natural humic acid and all compounds capable of substituting for it show an induction period, measurable in terms of hours, before stimulation is exerted.

(d) Humic acid does not act by increasing directly the availability of constituents normally added to, or present in, the medium, *viz.*, glucose, oxygen, free or fixed nitrogen, carbon dioxide, Ca, Mg, Na, K, Fe, PO_4, Cl, SO_4, HCO_3; or by deactivating toxic metabolic products; or by affecting the surface tension, viscosity, potential differences between culture medium and organism, or oxidation-reduction potential of the medium.

(e) Under conditions where humic acid exerted no stimulative effect the iron substitution compounds likewise exerted no effect, even though other chemical substances and physiological conditions could at the same time increase the growth considerably.

The only observed inherent difference between various types of iron compounds obtains with respect to the physiological induction period. In the case of *Azotobacter,* natural and synthetic humic acids ordinarily commence to exert stimulation 3 to 8 hours after addition, organic acid iron compounds after 8 to 15 hours, and inorganic iron compounds after 15 to 30 hours. Natural and synthetic humic acids are to be classed, therefore, as stimulants which provide iron for growth and nutrition in a form *more highly available* than that generally supplied in media. They are more highly available than organic acid iron compounds by virtue of induction period differences; than inorganic iron by virtue of both induction period differences and solubility relations. They may also be classed as nutrients, since, as pointed out above, when time is not a limiting factor, the same quantitative order of stimulation is obtained independently of iron compound type. Humic acids are not plant vitamins or any other constituents classifiable as essential to growth or nutrition, since they may be replaced by other substances containing iron.

It is realized that various humic acids, prepared from various soils or sugars and metals under various conditions might, contrary to our findings with *Azotobacter,* occasionally exert stimulation from various causes in addition to that of contained iron, *e.g.*, from contained aluminum, silica, phosphate, etc.; by adding a protective agent against poisons; by increasing the availability of medium constituents; or by adding a specific, stimulating, purely organic substance. It is conceivable, for instance, that some organism being studied might possess a special requirement for, say aluminum, in which case a natural or synthetic humic acid containing aluminum impurity might very well act by virtue of supplying aluminum, instead of, or in addition to, iron. So far as we are aware, no comprehensive, critical data obtain in favor of any of these suggested causes.

On the other hand, iron contained in humic acid may promote other processes besides growth. Many biological processes, such as respiration, nitrification, catalase activity, etc., probably require iron. We have recently determined that apart from its essential function in both the growth and respiration of *Azotobacter,* iron is also essential in the chemical mechanism of nitrogen fixation by this organism. Under certain conditions of relative iron starvation, natural or synthetic iron-containing humic acids may supply this iron, replacing the more customarily supplied inorganic or organic iron compounds. In this sense the humic acids may be said to influence directly the mechanism of fixation. Under ordinary conditions, humic acids have no such direct influence, but exert the same quantitative stimulation whether the organisms are grown in free or in various forms of fixed nitrogen.

In conclusion, a few of the implications of these findings, apart from their relation to respiration, growth, and nitrogen fixation by *Azotobacter,* may be mentioned. There are undoubtedly fifty or more different plant and animal growth stimulators described in the literature, such as bios, yeast water, plant extracts of various kinds, organic extracts, peat extracts, soil extracts, auximones, egg albumen, egg extract, Rhizopin, vitamin B and possibly other vitamins, in which the chemical reason for stimulation is in practically no case known. Humic acid is thus probably one of the few growth promoters of which the chemical constitution of the active constituent has been definitely determined. It is possible that many, perhaps the majority, of the promoters consist substantially of iron bound in an organic, highly available form, since their action appears to be very similar to that of humic acid; indeed, it may be suggested that the real problem in connection with the chemical nature of the active fraction of these promoters would perhaps more often be to show that they do *not* function chiefly because of contained iron. Many media, *viz.,* legume nodule bacteria media, stock culture media, are made up to contain yeast water, plant extracts, egg extract, or bios, of complicated and relatively unknown general composition; the use of humic acids, particularly synthetic ones, would perhaps often provide desirable substitutes in some degree avoiding this objectionable feature. It is probable, for instance, that the recent yeast-growth stimulant prepared by Fulmer, Williams, and Werkman[2] in the sterilization of synthetic yeast medium at elevated temperatures is similar to our synthetic humic acids, the iron being derived from the probable 1 p.p.m. iron impurity contained in the 5 per cent. sucrose employed.

It is possible that iron added as humic acid to water cultures of higher green plants would upon occasion be superior to most of the forms of iron now employed, especially in the matter of requiring much less frequent addition. Whereas inorganic iron compounds tend in general to precipitate as the pH is increased, the converse obtains with respect to humic acid, above pH 4. Even compounds like ferric citrate precipitate in neutral solutions, upon long standing, whereas properly prepared humic acids remain soluble indefinitely. Many higher plants grown in water cultures must be maintained under relatively acid conditions, in order to derive sufficient iron from the forms usually supplied; the use of humic acid might permit satisfactory or improved growth under substantially neutral or alkaline conditions.

The nutritional value of humus and soil organic matter in agricultural cropping is recognized generally, being ascribed, however, chiefly to indirect effects upon soil moisture, texture, aeration, etc. How far the nutritional value is directly referable to rendering iron more available, particularly in soils in which the inorganic iron solubility is low, is an important problem of agricultural science which would appear to be in some measure enlarged by the present investigations. It is our hope, in view of the fact that the latter have been concerned solely with *Azotobacter,* that the general problem of the influence of natural and synthetic humic acids upon the growth and nutrition of higher plants under field and water culture conditions will receive the attention of plant physiologists equipped to deal with the question.

DEAN BURK
HANS LINEWEAVER
C. KENNETH HORNER
F. E. ALLISON
FERTILIZER AND FIXED NITROGEN INVESTIGATIONS UNIT, BUREAU OF CHEMISTRY AND SOILS, UNITED STATES DEPARTMENT OF AGRICULTURE, WASHINGTON, D. C.

[2] *J. Bact.*, 21, 299, 1931.

BOOKS RECEIVED

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