the determination of ages of various carbonaceous materials in the range of 1,000–30,000 years.

This investigation is continuing with other sources of carbon and is being extended to other possible cosmic radioelements. A more detailed report will be published elsewhere.

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Fowl Spirochetosis Transmitted by *Argas persicus* (Oken), 1818 From Texas[1]

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Hoffman, Jackson, and Rucker (*4*) have given a preliminary report of spirochetosis in turkeys in California in which no vector was found, although a careful search was made in all poultry houses. This spirochetosis manifested itself in a mild nature compared with that reported by various investigators in other parts of the world. The symptoms were: standing or sitting with the eyes shut, anorexia, loss of weight, and diarrhea. More severe cases walked with difficulty. In a later paper, Hoffman and Jackson (*3*) reported the pathological effects of the spirochete in fowl. The epizootiology of the disease in the flock remained unknown.

Steinhaus (*7*) reported the isolation of an unidentified spirochete from hen's eggs after inoculation with liver tissue from hens raised in Montana. This spirochete was not pathogenic for chickens—a fact which may indicate a difference from the known infectious agent of fowl spirochetosis.

Marchoux and Salimbeni (*6*) showed that the common fowl tick, *Argas persicus*, is a vector of *Borrelia anserina* (Sakharoff) (= *Spirochaeta gallinarum* Blanchard), the agent of fowl spirochetosis. This was verified by the work of Balfour (*1*) and others. The spirochete is transmitted to the progeny of an infected tick through the egg. Incubation in the fowl, when tick transmitted, takes 4–9 days before spirochetes are demonstrable in the peripheral blood. Recovery from the disease is followed by lasting immunity, but in some outbreaks in the Old World the case fatality has been from 60–90 per cent.

Cooley and Kohls (*2*) report *Argas persicus* of almost world-wide distribution in warm climates and a vector of avian spirochetosis in many Old World regions and in Brazil, Panama, and Cuba in the New World. Hungerford and Hart (*5*) showed that the common red mite of chickens (*Dermanyssus gallinae*) can serve as a vector of the fowl spirochetosis.

In the present studies a white Leghorn rooster, on which a large number of ticks obtained from a poultry raiser in El Paso, Texas, were being maintained, became very ill. Blood smears were not made at first, and later they were negative for parasites. Several hundred progeny from these ticks and a few unfed nymphs and adults were next fed on a white Leghorn pullet. A blood smear from the normal hen was negative with Giemsa stain. In 6 days the pullet was obviously ill, and blood smears were positive for spirochetes. On the 7th day many spirochetes were present in blood smears, and a few were still present in the peripheral circulation on the 8th day. After the 8th day no spirochetes were found in blood smears.

Symptoms of the spirochetosis were: jaundice, anorexia, and diarrhea, with loss of weight. The rooster exhibited symptoms of partial paralysis. Both birds tended to sit and droop the head with the eyes shut. Recovery was uneventful in each case. It is believed that this is the first finding of tick-borne avian spirochetosis in the United States.

[1] Paper No. 2338, Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul.

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A Growth Inhibitor and a Growth Promotor in Sugar Cane[1]

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In connection with a study of structural development in sugar cane, a series of investigations was begun on the growth-regulating substances produced by that plant. In order to find a method by means of which the substances from all tissues of the plant, chlorophyllous as well as nonchlorophyllous, would be extracted, several methods of extraction and analysis described in the literature were tried. The results were unsatisfactory, however. When the direct ether extraction method of Boysen-Jensen was used, the chlorophylls, easily extracted from the green tissues of sugar cane, caused undesirable coagula which interfered with the accurate assay of growth substance in the *Avena* test. Van Overbeek, *et al.* (*10, 11*) applied this method to node and internode tissue of sugar cane stem, where apparently little or no chlorophyll was extracted. Furthermore, in all instances in which tissue was extracted with ether or with water, the sets of coleoptiles showed mixtures of curvatures, some positive toward the agar block and some negative. Since it was thought that such curvatures could not be averaged to give results of any significance, a series of experiments was begun to determine the source of the positive curvatures.

The presence in plants of substances which prevent the

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growth of the *Avena* coleoptile and cause positive curvatures in the *Avena* assay has long been suspected, but only in relatively recent years have direct evidences of the production of at least one substance been obtained. These evidences indicate that growth-inhibiting substances are produced in a large number of plants and perhaps in all plants. They seem to be produced in quantities varying in relation to the quantities of growth-promoting substances; they may cause little or great interference in growth substance assays and may easily be overlooked when present in small quantities.

Goodwin (*4*) reported evidence for the presence of inhibiting substances in ether extracts of plant tissue. Diffusion coefficients of auxins extracted by ether did not agree with the theoretical coefficient for auxin a or b. He concluded that this discrepancy was due not to an auxin of different molecular weight but to substances which partially masked the biological effect of auxin on the *Avena* coleoptile.

Stewart (*9*) found a substance in the cotyledons of radish plants which caused positive curvatures of oat coleoptiles. The inhibitor reduced the degree of negative curvatures when extracts of it were mixed with 3-indole acetic acid. Stewart concluded that the *Avena* test would give curvatures that would be correct measures of neither auxin nor inhibitor if both were present in the extract.

Van Overbeek (*10*), using direct ether extraction of nodal and internodal tissue of sugar cane, found that heating tissues in boiling water caused the liberation of a substance that had an inhibitory effect on the curvature of the *Avena* coleoptile. Fresh tissue frozen with solid carbon dioxide and then extracted with ether appeared not to yield the inhibitor.

Because Avery, Berger, and White (*1*) and Shalucha (*8*) have been able to obtain high yields of growth-promoting substance from several kinds of plants with the use of aqueous extraction of lyophilized leaf tissue, and because DuBuy (*3*) obtained good results with water extracts of tissue frozen with solid CO_2, it was decided to try water as primary extractant. It was decided also to attempt two ether extractions, one from neutralized water extract and another subsequent one from the same water extract acidified. A single test on ether extraction from acidulated distilled water yielded a majority of positive curvatures in our sets of coleoptiles. At about this time Larsen (*7*) published the results of his work leading to the identification of 3-indole acetaldehyde as a neutral growth substance, inactive in the *Avena* test, that could be converted to 3-indole acetic acid by action of soil or of the Schardinger enzyme of milk.

The method now used, after several modifications, is given below. No experiments to determine the thoroughness of extraction have been performed, nor have attempts to identify the substance been made. The data are presented as averages of degrees of curvatures of the coleoptiles and are to be considered at this stage of the investigation as qualitative only.

Materials and Methods

The plant material used was taken from nodal and internodal tissue and young leaves of a sugar cane hybrid.[2] Stem material consisted of internodes within or, in some experiments, just below the spindle cluster. Leaf material was comprised of blade tissue of leaves 3, 4, and 5, numbering from the first visible leaf of the spindle cluster (numbering method of Clements, *2*).

The "deseeded" *Avena* assay method of Skoog was used, with two decapitations and a 5-hour photographing period. All work with the coleoptiles proceeded in a specially constructed dark room in which temperature was maintained at 25°–26°C. and relative humidity at 90–91 per cent. Light was supplied by neon in ruby glass tubing mounted over the work table. This provided bright red light of wave lengths above 6,000 A.

The first experiments were carried out utilizing only 50 or 100 mg. of fresh tissue. This quantity was increased weekly until a sample of 200 grams of fresh weight produced significant curvatures in the *Avena* test. All subsequent extractions were made on 200 grams of fresh tissue. The tissue was treated as follows:

(1) The sample, of green or of nonchlorophyllous tissue, was cut as rapidly as possible into pieces 4 mm. or less on a side. The cutting process was performed on a slab of solid CO_2, which not only accomplishes quick freezing but also facilitates cutting succulent tissue.

(2) The sample was separated into approximately equal quantities, each quantity then being placed in dry ice and kept in the frozen state for approximately 20 hours. The quick freezing seemed to facilitate extraction of the growth substances.

(3) The jars were opened, the frozen tissue covered with distilled water (pH 5.5) at room temperature, and the sample muddled with a glass pestle or thick stirring rod, a few small pieces of dry ice being added from time to time to create a carbon dioxide atmosphere. This ostensibly reduces the chances of possibly undesirable oxidation. It also markedly reduces the tendency of the tannins to oxidize to pigmented compounds, and the water is agitated by the bubbling of the CO_2 gas, thereby effecting a constant stirring.

(4) After two hours the water was decanted into a Buchner funnel, and more distilled water was put on the sample and allowed to extract for one-half hour more. The water was decanted into a Buchner funnel, and the residue was washed once, squeezed by hand through a double thickness of cheese cloth, and placed in another sample bottle for further treatment as desired.

(5) The water extract was filtered in several flasks, the number depending upon the volume of water. With the large samples used in these experiments the water extract was usually between 800 and 1,000 ml. in volume when the filtrates were combined and the flasks rinsed with distilled water.

(6) The water extract was then neutralized with 8 per cent (saturated) $NaHCO_3$ solution. No glucose was added because of the high sucrose content naturally resulting from the cane tissue. Between 5 and 10 ml. of bicarbonate is usually sufficient, and the solution, when checked with the Macbeth pH meter, reads pH 7–7.3.

(7) The neutralized extract was placed in separatory funnels and covered with approximately equal volumes of freshly distilled diethyl ether. Emulsifying substances, usually finely suspended particles of tissue, were sometimes included in the water extract, necessitating use of a swirling motion and avoidance of shaking. It is often unavoidable that a coagulum

[2] *Saccharum officinarum* variety H-32-8560 crossed with unknown pollen. The seedlings were supplied through the courtesy of A. J. Mangelsdorf, Experiment Station, Hawaii Sugar Planters' Association.

containing ether results; when this occurred, the coagulum, which formed on top of the ether layer, was drawn off last and centrifuged to remove ether. The centrifuged residue was washed again with ether and the ether added to the larger ether extract. After the water was drawn off, the ether was allowed to run into a beaker. The water was then put back to the separating funnel and the process repeated. After a third repetition, the three ether extracts were combined, making the neutral fraction.

(8) To the water, which increases somewhat in volume by solution of some ether, was added, with stirring, a quantity of 15 per cent tartaric acid. The electrodes of the pH meter were kept in the extract, acid being added until pH 4 was reached. The water extract was then ether-treated as described in (7).

(9) Each ether extract was evaporated to 1 ml. in water by placing the beaker on a hot plate with very low heat and directing the current of air from an electric fan across the open top. During evaporation the ether was transferred successively into small beakers and finally into shell vials marked for 1-ml. volume. Each beaker was rinsed with a small quantity of ether. If there was not enough residual water, distilled water was added to make a volume of 1 ml.

(10) The 1-ml. extracts were combined in the vials with 1 ml. of 3 per cent agar, warmed sufficiently to be fluid, the solution then being mixed by gentle shaking and poured into a mold, constructed (of glass microscope slides glued to a lantern slide glass) to permit adjustment of the agar level to a 2-mm. depth. Rectangles of the gelled agar were cut in the cutting frame with a razor blade into cubes of 8-mm.^{3} volume, for use in the *Avena* test.

Results

In all experiments in which this procedure was followed, mixtures of significant curvatures were obtained. Representative results are given in Tables 1 and 2.

TABLE 1

Curvatures Obtained With 200 Grams Fresh Sugar Cane Stem Immediately Below Spindle Cluster*

	No. of coleoptiles	Degrees maximum curvature	Degrees minimum curvature	Average
Experiment 1				
Acid fraction	12	+9	+3	+6.7
Neutral fraction	12	−23	−4	−9.3
Experiment 2				
Acid fraction	14	+24	+6	+13.7
Neutral fraction	17	−11	−2	− 4.2
Experiment 3†				
Acid fraction	22	+8	+2	+ 4.8
Neutral fraction	24	−21	−7	−12.4

* Different samples of sugar cane of mixed and various ancestry were used in each experiment. The data may therefore not be comparable.

† Part of an experiment with young stem tissue.

Although no other tests were made to substantiate the data of Table 2, it appears that with the material used there is evidence of a much greater amount of inhibitor than of growth promotor. These tissues were obtained from upright, growing sugar cane with dormant axillaries. No tests have been made with regard to thorough or prolonged extraction, and the assumption that our data represent extraction of free growth substances is based upon the results of Van Overbeek's experiments (*10*), where it appears that most of the free auxins "are extracted within the first half-hour."

The growth-inhibiting substances in Table 1 are in approximately the same concentration as those in Table 2, but the growth-promoting substances are in much greater quantity than indicated in Table 2; and in Table 1 (Experiments 1 and 3) the growth-promoting substances are in greater concentration than the growth inhibitors in the same plant material.

Conclusions

No attempts have been made to report these data on bases of total degrees curvature, indole acetic acid equivalents, or any of the other units found in the literature. The method used is not yet developed to the stage where accurate, reproducible results may be obtained. It is our opinion that until a better knowledge is gained of the substances being dealt with in sugar cane, reporting of results on a quantitative basis has little significance. The material used to date was taken from hybrid cane the male parent of which is not known; the stand is therefore made up of many strains, often evidenced by distinct color differences, hairiness, and other superficial characters. The quantity of growth substances may vary among plants, and the relative proportion of the two kinds may vary within the plants. Future studies will be made upon plants of one variety.

TABLE 2

Curvatures Obtained With 200 Grams Internode, Root Band, and Intercalary Meristem Tissues of Sugar Cane

Tissue	Fraction	No. of coleoptiles	Degrees maximum curvature	Degrees minimum curvature	Average
Internode	Acid	18	+9	+3	+4.7
	Neutral	22	−7	−1	−3.1
Root band	Acid	19	+15	+2	+6.2
	Neutral	15	−5	−1	−1.6
Intercalary meristem	Acid	14	+6	+2	+3.6
	Neutral	18	−4	−1	−2.4

The growth substances of a plant form a complex system not analyzable by a simple procedure. At least three groups or categories are now known. These are: (1) growth-promoting substances, which produce negative curvatures in the *Avena* test. Auxenolonic acid, auxentriolic acid, and 3-indole acetic acid are known to be produced in higher plants; (2) neutral growth substances, the presence of which in plants has been demonstrated by Larsen (*6*, *7*) who identified one as 3-indole acetaldehyde and has evidence that this substance, inactive in the *Avena* test, is converted by aldehydrases to 3-indole acetic acid; and (3) growth-inhibiting substances, which produce positive curvatures in the *Avena* test. Presence of this kind of growth substance has been demonstrated by Larsen (*6*), Kisser (*5*), Stewart (*9*), and others in tissue which apparently was relatively low in growth-promoting substances. The nature of these compounds, or their relationship to the others, is not yet known, but Stewart (*9*) presents evidence indicating that the inhibitor of radish cotyledons may be hydrolyzed to growth-promoting substance.

It is clear, then, that a satisfactory extraction technique must somewhere provide for the separation of the *two* groups of growth substances which are active in the *Avena* test. Unless this is done, the *Avena* assay can only approach reasonable accuracy when either group of substances is extremely low in relation to the other.

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Comparative Nutritive Value of Casein and Lactalbumin for Man

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The classic work of Osborne and Mendel (*5*) showed that casein is deficient in cystine and that its companion protein, lactalbumin, is superior in promoting the growth of rats. This finding was promptly (and at that time, perhaps, properly) used in partial explanation of the superiority of breast milk over cow's milk in the feeding of infants (*2*). In confirmation of these early observations, we have recently reported (*1*) that the addition of methionine to an enzymic hydrolysate of casein (amigen) resulted in an accelerated rate of growth in rats, and when the supplemented hydrolysate was given intravenously, it improved nitrogen retention in dogs. When the same type of supplementation studies were made in humans, there was no improvement in nitrogen retention. Four groups of humans were employed: (1) surgical patients fed intravenously, (2) normal infants, (3) normal adults on a maintenance nitrogen level, and (4) normal adults who were protein depleted and fed just enough nitrogen to supply the approximate endogenous needs. Methionine supplementation did not increase the efficiency of nitrogen utilization from the casein hydrolysate.

These findings naturally led us to determine the comparative effectiveness of intact casein and of lactalbumin in promoting nitrogen retention *in man*. Four normal adults were placed on a protein-low diet (*4*) for 12 days, until a constant nitrogen excretion (assumed to be approximately endogenous) had been reached. The nitrogen intake during all depletion periods was 0.008 gram/kg. body weight/day, and for the last 4 of the initial 12 days the average nitrogen loss was 3.22 grams daily. The caloric intake during the entire 40-day study was maintained constantly at 40 calories/kg. body weight.

Four-day periods of supplementation with either casein or lactalbumin were then alternated with 4-day depletion periods. The two proteins were fed alternately, and each was administered for two 4-day experimental periods in order to minimize the effect of antecedent conditions on nitrogen balance. A level of supplementation insufficient to produce positive nitrogen balance was intentionally chosen. This level was 0.033 gram of nitrogen/kg. body weight as the test protein, equal to 2 per cent of the total calories. The average nitrogen balances for each subject for each period are given in Table 1. Subject C contracted a mild type of prevalent "influenza" and could not complete the test.

TABLE 1

NITROGEN BALANCES FOR EACH PERIOD

(Data expressed in grams nitrogen/day)

Periods (4 days each)	Subject				
	A	B	C	D	Avg.
Depletion	−3.92	−2.26	−3.81	−2.90	−3.22
Casein	−.76	−.64	−.83	−.93	−.79
Depletion	−4.13	−2.88	−3.52	−3.00	−3.38
Lactalbumin	−.65	−.47	−.69	−1.40	−.80
Depletion	−3.48	−2.29	−2.81	−2.75	−2.83
Casein	−.74	−.32	−.87	−.79	−.68
Depletion	−4.09	−2.10	—	−2.34	−2.84
Lactalbumin	−1.44	−.05	—	−.66	−.72

These experiments reveal no significant difference between the two proteins in maintaining nitrogen balance in man. The average endogenous value for the four depletion periods was −3.07 grams. The addition of casein spared 2.33 grams of body nitrogen, and the addition of lactalbumin, 2.31 grams. These average values are closer than might again be encountered, but the individual balances were within the range of variation that we have observed in this type of study. All pertinent details of the study will be published elsewhere.

We have approached the calculation of "biological value" with temerity, since, in our experience, such comparative values more often mask than disclose information. Using an average for the four depletion periods as the endogenous level of urinary and of fecal nitrogen excretion, average "biological values" are: for casein, 89.0 and 94.7, respectively; for lactalbumin, 90.2 and 93.3. In spite of the similarity in these final average figures, it should be noted that the variation in the individual values is as great as that observed in experimental animals (*3*).

We have attributed the failure of methionine to improve the nitrogen retention of a casein hydrolysate in man to the fact that man is not covered with hair. Hair contains a high percentage of cystine, and it is logical that the requirement of the rat and the dog for sulfur-containing amino acids should be greater than that of man (*1*). Whether or not this explanation is valid, these data demonstrate that lactalbumin is not superior to casein in promoting nitrogen retention in man.

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