

In the calorimetric experiments referred to the energy expense of utilization of body nutrients katabolized was tentatively found, as an average of five determinations, to be equivalent to 14.4 cal. per kilogram of live weight during fast and equal time standing and lying, or to 12.6 calories per kilogram during fast in the lying position alone, or to 26.5 per cent. of the metabolizable energy of the oleo oil and dried beef muscle fed to represent the body nutrients katabolized.

To demonstrate the significance of the theoretical base value of energy metabolism, a graph is presented (Fig. 1) representing previously determined curves of

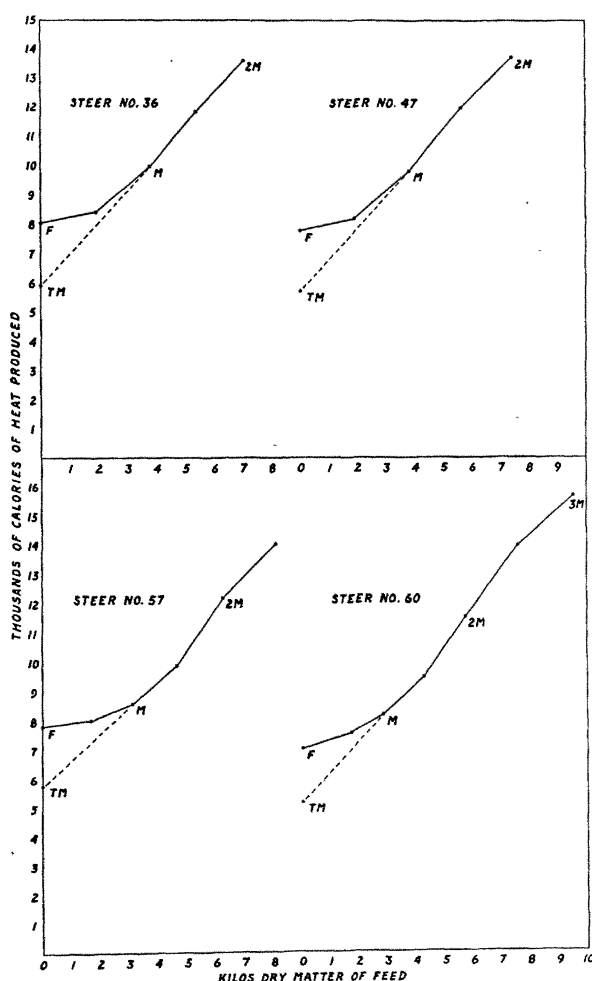


FIG. 1. Relation of heat production to food consumption, as observed and as corrected for dynamic effect of body nutrients katabolized. M = maintenance, F = fast, and TM = theoretical minimum heat production.

heat production in relation to food consumed with four steers, (1) as observed, and (2) as corrected by the use of the recently determined factor for computing the dynamic effect of body nutrients katabolized.

The curve of heat production in relation to the energy of the food, so corrected, becomes nearly a straight line up to the moderately high level at which the metabolizability of the food begins to diminish, thus providing a basis for the expression of energy values of diets, and energy requirements of animals, both for maintenance and for body increase in the same terms, presumably without extensive error.

While it is obviously impossible directly to measure this theoretical base value of heat production, as defined, the indirect estimation which is presented serves at least to illustrate the point of view that there is such a value, and that dynamic effects of nutrients as directly observed at planes of nutrition below that of energy equilibrium are fundamentally invalid.

The maintenance heat production, therefore, is considered the most nearly correct base value from which to measure dynamic effects in nutrition.

It is impossible to make a full statement of the philosophy of this matter in so short a communication, and a detailed discussion will be presented elsewhere.

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ESTIMATION, ISOLATION AND IDENTIFICATION OF AUXINS IN PLANT MATERIAL

FOR estimation and concentration of auxins the following methods have been used, namely, the diffusion method,¹ the extraction process,² the enzymatic method (lipase,³ chymotrypsin^{3a}), the alkaline alcoholic hydrolysis⁴ and the biological digestion method.⁵

The biological digestion method of Kögl, Haagen-Smit and Erxleben as performed by feeding experiments on humans is capable of giving the total hormone content, both the free hormone and the precursor form (or bound form) as well. By necessity this method is limited in its application.

Using the results obtained in the biological digestion method as a criterion for the assay of the total growth hormone set free in dormant tissues, the following more practical method was developed, whereby one is able to remove an amount which is equivalent to the amount of the growth promoting substances obtained by the biological method. This modified procedure has been in use in this laboratory for the past two years in the investigation of auxin-like substances in high

¹ F. W. Went, *Rec. Trav. Bot. Neerl.*, 25: 1-116, 1928.

² G. S. Avery, *Am. Jour. Bot.*, 26: 679, 1939; K. V. Thimann and F. Skoog, *Am. Jour. Bot.*, 27: 951-960, 1940.

³ F. Kögl, A. J. Haagen-Smit and H. Erxleben, *Zeits. physiol. Chem.*, 220: 137-161, 1933.

^{3a} F. Skoog and K. V. Thimann, *SCIENCE*, 92: 64, 1940.

⁴ F. Kögl, A. J. Haagen-Smit and H. Erxleben, *Zeits. physiol. Chem.*, 225: 215-229, 1934.

and low protein wheats and in the isolation work from corn.

The procedure is briefly as follows: Dry whole wheat kernels are soaked for four hours in sodium hydroxide solution at pH 10.5, ground in a glass mortar with clean sand, allowed to stand at 20° C. for 45 hours (together with one ml of toluene) with the pH maintained at 10.5. At the end of this period, the mixture is centrifuged, and the supernatant liquid is tested by the standard *Avena* procedure.

Table I illustrates a typical experiment. The value obtained at pH 10.5 compares favorably with the amount of auxin recovered by the biological digestion method.

TABLE I

pH	Auxin per kilogram of wheat*
4.0	0.37 mgm.
7.0	2.25
10.5	6.96
11.5	1.50

* In terms of indole-3-acetic acid.

Temperature effects were also considered. Above 60° C. losses began to occur. The maximum rate of hydrolysis was found to lie between 35° and 40° C. However, since the rate was almost the same at 20° C., the investigation was carried out at room temperature in view of practical considerations involved.

Tests upon samples of corn showed the same effects of increased auxin activity upon standing at pH 10.5 as did the wheat. Yellow cornmeal, fresh from the mill, was found to be more convenient for isolation purposes and accordingly was used.

It was found that a 50 per cent. acetone-water mixture could be satisfactorily used for the hydrolysis. The ratio of solvent to cornmeal was adjusted to form a thick paste-like mass, and the pH was maintained near 10.5 for a period of 45 to 60 hours. After the hydrolysis period, the mass was pressed, and the residue was washed with a 50 per cent. acetone-water

mixture. The filtrate, combined with the washings, formed the initial extract for the isolation. Addition of sodium chloride gave rise to the formation of a separate acetone layer; after separation, the aqueous residue was washed with acetone. The acetone solutions were distilled, and the distillation residues were combined and extracted with ether. The isolation proceeded from the ether-soluble material according to the general procedures already described in the literature for isolation from other materials.^{4, 5}

The isolations gave as an end product a syrup which was physiologically active and which gave a color reaction with ferric chloride similar to that given by indole-3-acetic acid. Upon long standing this syrup crystallized. A portion of the material, when recrystallized, showed a melting point of 164–5° C. and no melting point depression on mixing with synthetic indole-3-acetic acid. This is proof of the identity of the isolated material with indole-3-acetic acid, and is the first time that indole-3-acetic acid has been isolated from higher plants. Since the amount of the isolated acid is many times greater than the auxin amount indicated by the normal extraction process, it undoubtedly represents a large part of the auxin present in bound form.

In addition to the indole-3-acetic acid, there was obtained a small amount of crystalline pseudo-auxin-a which melted at 196–7° C., with sintering beginning at 173°. Kögl, Koningsberger and Erxleben⁶ give the melting point of pseudo-auxin-a as 193–4° C., with sintering beginning at 176°. This shows the presence of some auxin-a in the original starting material, which rearranged to pseudo-auxin-a during the isolation procedures.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

AN IMPROVED GAS BURNER FOR SMOKING KYMOGRAPH PAPER

WHILE experimenting with a protective "skirt" in order to decrease the susceptibility of the paper-smoking flame to air drafts, it was found that such a protective skirt can be made to yield very great improvements in other characteristics of the flame as well. Not only are draft sensitivity and flickering greatly diminished, but the flame also becomes far more efficient in smoke formation, becoming relatively cool, dull red and very smoky. The relative coolness has been found particularly valuable in the student laboratory,

since we have found it practically impossible to seorch the paper with the new burner while using benzol enriched gas.

The essential improvement of the burner consists in surrounding the usual perforated delivery tube with a "skirt" in the form of an inverted V, open at the top and bottom, which serves the purpose of limiting the air supply to the flame. The amount of air drawn

⁵ F. Kögl, A. J. Haagen-Smit and H. Erxleben, *Zeits. physiol. Chem.*, 214: 241–261, 1933.

⁶ F. Kögl, C. Koningsberger and H. Erxleben, *Zeits. physiol. Chem.*, 244: 266–278, 1936.