about one fifth as rapidly, as measured by the rate at which o-phosphoric acid is split off.[1]

That adenine (in nucleotide combination) is the source of the ammonia formed in muscle during contraction has been amply demonstrated by Embden and his collaborators. The physiological significance of this important work is presumably quite unaffected by the fact, which we now have to report, that myoadenylic acid is not a normal constituent of muscle (except perhaps in traces), but a decomposition product. Our first intimation that this might be the case developed from the observation that when a protein free muscle filtrate is treated with an alkaline solution of calcium chloride[3] a large part of the purine nitrogen comes down in the precipitate. The calcium salt of myoadenylic acid is soluble in water, and should consequently—if present—remain dissolved under these conditions.

The purine derivative precipitated by calcium has been isolated by (1) precipitation with mercuric acetate in the presence of 2 per cent. acetic acid, followed (after removal of the mercury) by (2) precipitation with calcium chloride and alcohol from hydrochloric acid solution, which yields an acid calcium salt. By repeating the entire process the acid calcium salt is finally obtained as a microcrystalline precipitate. The yield is not far from quantitative, and accounts not only for most of the purine nitrogen of muscle, but also for most of the acid soluble phosphorus not present as o-phosphoric acid, phosphocreatine or hexose monophosphate. In the case of cat muscle the yield of purified material may be the equivalent of nearly 50 mg of phosphorus per 100 gm of muscle.

The acid calcium salt is not well suited for analytical purposes, owing to the difficulty of removing all the water. It may, however, be converted to a silver salt—by precipitation with silver nitrate from nitric acid solution—and the composition of this product has been found to be $C_{10}H_{18}O_{13}N_5P_3Ag_3$. It contains, in addition to adenine and carbohydrate, three molecules of phosphoric acid, or two more than in adenylic acid. Two of the three molecules of phosphoric acid are readily removed by hydrolysis with acid, and this fact is doubtless sufficient to explain why Embden and Zimmerman[2] obtained a nucleotide (myoadenylic acid) which still retains the one resistant phosphoric acid group.

The new substance includes also the phosphorus which Lohmann[4] believes to be present in the muscle in the form of pyrophosphate, but whether or not it is an ester of pyrophosphoric acid remains to be determined.

II. LIVER

The greater part of the organic acid soluble phosphorus of liver may be precipitated from the protein free filtrate, after removing the inorganic phosphate with alkaline calcium chloride solution, by the addition of alcohol. Purification by dissolving in water and reprecipitating with alcohol finally yields a calcium salt, crystallizing in spherulites or aggregates of short needles, and having the composition $C_3H_7O_6PCa.1\frac{1}{2}H_2O$. It is the calcium salt of glycerophosphoric acid, which in spite of text-book statements has not—as far as we have been able to determine—been isolated from animal material before, at least under conditions which preclude its formation from lecithin and related substances.

Experiments are now under way which it is hoped will lead to some procedure for the quantitative estimate of free glycerophosphate in tissue filtrates. From present indications this substance appears to account for at least one third of the total acid soluble phosphorus of the liver.

The properties of the above-mentioned calcium salt, together with the fact that it gives an intense greenish blue color on applying the Denigès codeine test after oxidation with bromine,[5] and forms no insoluble double salt with barium nitrate,[6] identify it as α-glycerophosphate. This is of particular interest since all preparations of lecithin so far examined by means of these tests have been found to contain mainly the β form of glycerophosphoric acid.[7]

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BALANCED FERTILIZERS AND LIEBIG'S LAW OF THE MINIMUM

LIEBIG maintained that "by the deficiency or absence of *one* necessary constituent, all others being present, the soil is rendered barren for all those crops to the life of which *that one* constituent is indispensable."[1] This afterwards became known as the "law of the minimum," from which it must logically follow, as indeed Liebig himself announced,[2] that

[3] This is the first step in the isolation of phosphocreatine (C. H. Fiske and Y. Subbarow, *J. Biol. Chem.*, 81: 629, 1929) where it serves the purpose of removing inorganic phosphate and other products.

[4] K. Lohmann, *Biochem. Z.*, 202: 466, 1928; 203: 164, 172, 1928.

[5] O. Bailly, *Ann. chim.*, 6: 96, 1916.

[6] P. Karrer and H. Salomon, *Helv. chim. acta*, 9: 1, 1926.

[7] O. Bailly, *Ann. chim.*, 6: 215, 1916; P. Karrer and P. Benz, *Helv. chim. acta*, 10: 87, 1927.

[1] Justus Liebig, "Chemistry in Its Applications to Agriculture and Physiology," third edition, 1843.

[2] Justus Liebig, "Les lois naturelles de l'Agriculture," 2, second chapter, 1863.

nutrient elements are absorbed in certain definite proportions ("chiffres proportionals") in such a manner that a limit having been reached by one, the absorption of the others is retarded (depressed) *ipso facto*.

Accordingly, if with time as the abscissa and the quantity of nutrient element absorbed during the growth of a plant as the ordinate, graphs are plotted indicating the course of absorption of the three principal fertilizer constituents, nitrogen, phosphorus and potassium, from a soil to which a "complete" fertilizer has been added, it will follow from Liebig's basic assumption that, with all other growth factors constant, the graph showing the course of absorption of *any one* of these elements from a fertilizer containing only two of them would be displaced from its former position and will now fall below it.

The observed facts, however, were not always in harmony with Liebig's hypothesis. For example, from Liebig's assumption it should follow that the relationship between the supply of a particular plant nutrient and the dry matter produced would be one of simple proportion, and indeed this was Liebig's conception.[2] But this is not the case,[3, 4, 5] for the curve of dry matter produced plotted against nutrient supply is sigmoid in shape and not a straight line having the x and y coordinates equal to one another as would be expected if Liebig's deduction held true.

Liebscher[6] suggested the substitution of his "law of the optimum" for the "law of the minimum." In effect, this is that the plant "is able to utilize to a greater extent that growth factor present in minimum amount the more the other factors already present approach toward their optimum values." But since water was the only growth variable tested by Leibscher, generalizations are hardly possible.

A few years later Mitscherlich[7] announced his "Gesetz der physiologischen Beziehungen," according to which any one individual growth factor can, independently, increase the growth of all others, provided it is not already present in optimum amounts. Mitscherlich's "law" has been variously interpreted. Some authorities have argued that it furnishes additional support for Liebig's "law of the minimum" and others consider that Liebig's and Mitscherlich's views are irreconcilable.

It has been argued[8] in favor of the retention of the "law of the minimum" that as shown by the experiments of Henrich,[9] Langer[10] and Lemmermann[8] certain applications of the "law" have been of value in explaining to the farmer certain basic principles of manuring.

No direct proof, however, against Liebig's "law of the minimum" has been forthcoming until Lagatu and Maume,[11] of l'Ecole Nationale d'Agriculture de Montpellier, in a series of brilliant investigations begun in 1919, showed that the course of absorption of nitrogen, phosphorus and potassium in the vine was irreconcilable with Liebig's "law." Owing, possibly, to the inaccessibility of some of the journals in which these investigations have been published, they have, unfortunately, passed unnoticed in this country.

The investigations have centered upon the analysis of the leaves of vines from experimental plots treated with "complete" fertilizer (N, P and K) and with combinations in which one of each of the elements is absent. Plots having no fertilizer applied serve as controls. Analyses with respect to N, P and K were made at regular intervals from May to October of each year. They have found the following striking results:

(1) The application of an "incomplete" fertilizer deficient in one of the elements N, P or K results in an *increased absorption by the plant of the other two*.

(2) This lack of balance in a fertilizer containing two only of the principal fertilizer constituents (N, P, K) may be so great that the relative excess of these elements will "act as a brake" on the absorption of the one omitted to such an extent that *the plant will absorb from the soil containing this "incomplete" fertilizer less of the missing element than it would from the soil containing no fertilizer additions*.

(3) A lack of nutritional balance resulting from the addition of an unbalanced fertilizer results (except in the case of N deficiency) in decreased yields. This exceptional behavior of nitrogen is explained on the basis that this constituent is utilized more by the leaves than by the fruit (grapes). Development of the stems and leaves, however, will be retarded.

[3] A. Hellreigel and H. Wilforth, *Ztschr. des Vereins f. d. Rübenzucker-Industrie*, 1888; *Arb. Deut. Landw. Gesell.*, 34: 18, 1898.

[4] H. Weissmann, *Ztschr. Pflanzenernähr. u. Düngung*, 2A: 1–79, 1923.

[5] E. J. Russell, "Soil Conditions and Plant Growth," p. 36, fifth edition, 1927.

[6] G. Liebscher, *Jour. für Landw.*, 43: 49–216, 1895.

[7] Alfred Mitscherlich, *Landw. Jahrbücher*, 56: 71–92, 1921.

[8] O. Lemmermann, P. Hasse and W. Jensen, *Ztschr. Pflanzenernähr. u. Düngung*, B7: 49–98, 1928.

[9] R. Henrich, "Dünger und Düngen," Preisschrift, p. 2, third edition, 1896.

[10] L. Langer, *Jour. für Landw.*, 49: 209–229, 1901.

[11] H. Lagatu, "Rapport présenté au Congrès d'Agriculture régionale du Sud-Ouest," Toulouse, Mai, 1922, pp. 3–21; "Communication au Congrès de l'Association française pour l'avancement des Sciences," Montpellier, *Juillet*, 1922, pp. 22–31; *Compt. Rend.*, 172: 129, 1921. H. Lagatu and L. Maume, *Compt. Rend.*, 179: 782, 1924; *ibid.*, 179: 932, 1924; *ibid.*, 180: 1179, 1925; "Communication au Congrès des engrais azotés de synthèse à Montpellier," Juin 1, 1927, pp. 1–15; Extrait des *Compt. rend. l'acad. d'agric. France*, Avr. 6–Mai 11, 1927, pp. 1–24; *ibid.*, Juin 13, 1928, pp. 1–15; "Communication faite le 22 Juin, 1928, à Bordeaux au Congrès intern. du vin et du pin maritime," pp. 1–12.

Both (1) and (2) above are contrary to Liebig's "law." Lagatu and Maume's results show that whatever effect the absence of an element from a fertilizer has on the yield, *it is not due to a depression of absorption of the other elements, but, on the contrary, to a nutritional lack of balance due to increased absorption of the remaining elements.*

It will be of interest to know if the remarkable results obtained by Lagatu and Maume in the case of the vine will be found to be true also for other plants. This point is now under examination for apple-trees grown under controlled conditions.[12]

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EXPERIMENTAL INFECTION OF RATS WITH THE BALANTIDIUM FROM THE PIG[1]

THE following observations were made during an attempt to obtain an experimental infection of *Balantidium* in the rat, in order that some of the relations of this parasite to the diet of the host might be studied.

So far as the available literature shows, no previous attempt has been made to infect rats with the *Balantidium* from the pig. Pritze (1928)[2] says that Nöller (1926)[3] has raised the question as to whether or not the *Balantidium* of the Norway rat may have been acquired from the pig. Nöller's article has not been available, but with its exception it appears that no *Balantidium* has ever been noted in the rat. Scott (1927)[4] listed thirty-two species of this genus but not one of them from the rat.

Experimental infections of the *Balantidium* from the pig have been established in the monkey by Brumpt (1909),[5] by Hegner (1926)[6] and Rees (1927)[7] in the guinea-pig. According to Hegner (1927),[8] Ohi (1923)[9] was unable to infect the guinea-pig, rabbit and dog with pig material.

At the suggestion of Dr. H. L. Ratcliffe, 0.5 cc of a culture of the *Balantidium* from the pig, concentrated by centrifugation, was injected into the cecum of a rat, after laporatomy. This rat had been fed a stock diet, high in carbohydrate content, to which was added carrots. Examination of the cecum of this rat, which was killed two days later, revealed many balantidia, all trophozoites, including some dividing forms.

Attempts were made to infect a number of rats. Two methods were used and the material was derived from two sources. The first was to inject into the cecum of laporatomized rats under aseptic conditions, trophozoites of *Balantidium,* either from cultures which had been concentrated by centrifugation, or from the colon of the pig. In the second method, material from the colon of the pig was strained through cheesecloth. Rats were given five or six cubic centimeters of this strained liquid material by stomach tube. Material of this sort was used in the first method.

Results obtained by the two methods were as follows:

Source of material	Method of injection	No. animals injected	Positive for *Balantidium*	Negative for *Balantidium*
Cultures	Intracecal	22	9	13
Colon of the pig	"	14	2	12
Colon of the pig	Stomach tube	12	8	4
Total		**48**	**19**	**29**

These infections lasted from two to twenty-three days. Some of the rats were killed before examination which showed them to be positive, and it is quite probable that, had these rats been examined by operation and allowed to live, the infections would have persisted for a longer time.

The parasites were localized in the cecum of the rat. Dividing forms were found frequently. Daily examination of pellets obtained from the rectum of two rats, which were known to be positive, when carried on over a period of five days, failed to show any cysts. On one occasion trophozoites were found in a soft, mushy pellet.

Attempts to infect four rats with the cysts of *Balantidium* from the monkey were negative. Negative results were obtained in an attempt to infect four rats with the *Balantidium* from the guinea-pig.

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[12] W. Thomas and R. D. Anthony, *Proc. Am. Soc. Hort. Sci.,* pp. 81–87, 1926.

[1] From the department of protozoology, Johns Hopkins School of Hygiene and Public Health. This work was aided by a grant from the committee on scientific research of the American Medical Association.

[2] F. Pritze, 1928, *Zeit. f. Parasitenkunde,* 1: 345–415.

[3] W. Nöller, 1926, "Tierheilk. u. Tierzucht.," pp. 88–95.

[4] M. J. Scott, 1927, *Journ. Morph. and Physiol.,* 44: 417–453.

[5] E. Brumpt, 1909, *C. R. Soc. Biol.,* 67: 103.

[6] R. W. Hegner, 1926, *Amer. Journ. Hyg.,* 6: 593.

[7] C. W. Rees, 1927, SCIENCE, 66: 89–91.

[8] R. W. Hegner, 1927, "Host-Parasite Relations Between Man and His Intestinal Protozoa." New York. 184 pp.

[9] T. Ohi, 1923, *Taiwan Igakkai Zasshi,* 229: 19–20.