9626

Relation of Nicotinic Acid to Growth and Dermatitis Factors in Rice Polishings.

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(Introduced by L. Reiner.)

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Various extracts, adsorption and elution products of rice polishings and certain derivatives from yeast were investigated for the growth and dermatitis factors associated in nature with thiamin and riboflavin. These preparations from plant sources were tested with albino rats maintained on a purified sucrose diet,[1] supplemented daily with 20γ thiamin chloride (Merck) and 15γ riboflavin (Labco) and which exhibited cessation of growth and incipient symptoms of the nutritional deficiency.

The biological properties of these concentrates were standardized with growth rates, and curative responses established by depleted animals which received an aqueous acetone extract of rice polishings.* This preparation supplied 6 I.U. of vitamin B_1 in a daily dose of 75 mg. and biological assay or fluorescent examination proved it to be practically free of riboflavin although it is a satisfactory source of the other accessory factors in this group. Growth rates of 1.5-2.0 gm. daily over a period of 4 weeks were employed as a sensitive growth range and were preferred to higher rates which required a maximal response from the animals. Increased amounts of riboflavin (30γ level) have no significant effect on the growth rates which were similar to those obtained with 15γ daily.

An 80% butyl alcohol extraction of the rice polishings concentrate* conducted at room temperature yielded a 3-fold concentration of the essential growth factors, *i. e.*, 25 mg. of the viscous syrup (sp. g. = 1.4) were equivalent to 72 mg. of the rice polishings concentrate. Under similar conditions, a 75% dioxan extraction was more effective than a 90% dioxan extraction and the extractives equalled the butyl alcohol fraction in potency.

A dilute solution of the rice polishings concentrate was treated several times at pH 5 with Surrey fuller's earth. The primary adsorption removed thiamin and associated substances that possessed high adsorption coefficients and that equalled in weight the combined

[1] Cook, C. A., Clarke, M. F., and Light, A. E., *Science*, 1937, **85**, 503.

* "Ryzamin-B," Burroughs Wellcome Co. (U. S. A.) Inc.

substances adsorbed in the 3 subsequent treatments. The first adsorption product (B_1) was separated from the secondary adsorbates (II) and these latter fractions were combined and eluted with an alcoholic pyridine solution.[2] The pyridine was removed by repeated distillations in a partial vacuum and 20 mg. of the resultant hygroscopic brown powder was found to induce an average rate of growth (1.6 gm. per day) in depleted rats. The lesions of the florid dermatitis in rats[3] were healed with 20 mg. and 50 mg. daily of this material but the animals failed to regain a completely healthy appearance and their rate of growth was only 1.2 gm. per day. This was in marked contrast to the more rapid recovery observed with the butyl alcohol extract or the rice polishings concentrate. In no case where gross lesions had developed did the animals recover with the rapid growth which was typical of rats without dermatitis whose weight had been nearly constant for 10 days at the plateau period. In such animals, realimentation with the rice polishings concentrate produced a growth response of 2.3-2.6 gm. per day. The deficiency appeared more serious than when flavin was lacking and respiratory disorders were common in the diseased rats.

A primary adsorbate comparable to the International Standard (B_1) allowed a limited growth at 60 mg. levels when supplemented with riboflavin. The experimental animals required 38 days to exhibit a growth plateau in comparison with controls which received only thiamin chloride and riboflavin and displayed a characteristic plateau in 20-25 days (Table I). An alcoholic pyridine extract of this product at 12 mg. and 24 mg. levels provided similar evidence that a small amount of growth substances other than thiamin and riboflavin were present.

The filtrate from the multiple adsorptions proved to be inactive in 70 mg. doses when administered with only thiamin and riboflavin. Nevertheless, the findings of Lepkovsky, Jukes and Krause[4] were confirmed in that additions of 70 mg. of the viscous filtrate to the 20 mg. dose of the pyridine eluate previously assayed did produce a supplementary effect with a higher rate (2.2 gm. per day) of growth. The adsorption method did not completely separate the so-called filtrate factor and the silicates appeared to adsorb all factors to some extent.

An extraction of autoclaved 'Ryzamin-B' with 80% acetone pre-

2 Cook, C. A., and Carroll, R., *Ind. and Eng. Chem.*, 1936, **28**, 741.

3 György, P., *Biochem. J.*, 1935, **29**, 741.

4 Lepkovsky, S., Jukes, T. H., and Krause, M. E., *J. Biol. Chem.*, 1936, **115**, 557.

TABLE I.

Preparation	Growth g./day	Assay Period days
		Assays begin
20γ Thiamin chloride + 15γ or 30γ Riboflavin	*Plateau	at 25th day
72 mg. Rice polishings concentrate	2.3	28
90 " " " "	†2.6	28
5-10 mg. 80% Butyl alcohol extract	0.8	18
20 " " " " "	1.6	30
25 " " " " "	2.0	30
50 " " " " "	†2.2	21
50 " 80% Acetone extract of autoclaved rice polishings concentrate	0.8	24
20 " Pyridine eluate of adsorbates II	1.6	14
20 " " " " " "	1.2	21
50 " " " " " "	‡1.2	35
20 " Autoclaved pyridine eluate of adsorbates II	0.9	14
70 " Filtrate from II	no growth	14
70 " " " " + 20 mg. eluate of II	2.2	28
70γ, 140γ, or 1 mg. Nicotinic acid or amide	§no growth	
5 mg. Adenylic acid + 1 mg. nicotinic acid amide	" "	
25 " Rice polishings concentrate	1.2	7
25 " " " " + 70γ — 1 mg. nicotinic acid	1.0	32
25 " " " " + 1 mg. nicotinic acid amide	0.7	21
75 " " " " + 70γ — 1 mg. nicotinic acid	2.2	35
20 " Pyridine eluate of adsorbates II	1.2	7
20 " " " " " " + 70γ — 1 mg. nicotinic acid	0.9	32
20 " " " " " " + 1 mg. nicotinic acid amide	1.0	21
20 " " " " " " + 1-5 mg. adenylic acid or yeast nucleic acid	1.2	14

*A plateau period (± 4 g. in 10 days) ended depletion (average age of rats = 46 days) and preceded all assay periods.
†Lesions completely cured.
‡Symptoms partly cured.
§Lesions not cured.

cipitated much of the caramelized sugar but 50 mg. doses of the concentrated filtrate permitted a gain of only 0.8 gm. per day in the depleted animals. The alcoholic pyridine extract derived from the secondary adsorption products and previously assayed, was autoclaved for 8 hours at 120°C. The preparation in 20 mg. doses did not prevent the appearance of symptoms in some of the rats and allowed only a low rate of growth. These experiments supported previous observations with the autoclaved rice polishings concentrate that certain factors at different stages of their concentration could be altered by treatment at high temperatures.

The injection of growth hormone (anterior pituitary extract) in rats depleted of thiamin, riboflavin or the dermatitis factor failed to induce growth responses.

In view of the growth effects recently obtained with nicotinic acid and its amide,[5] these compounds were administered as supplements to animals receiving thiamin chloride and riboflavin, and to others which in addition were given preparations discussed above. The cereal grain included in the above ration[5] to provide suppletive amounts of the dermatitis factor makes comparisons difficult. The rice polishings concentrate and the extracts or eluates used in our experiments possibly contain nicotinic acid.†

Nicotinic acid (Eastman), 70γ, 140γ and 1 mg. daily, or nicotinic acid amide, 1 mg., did not stimulate growth in depleted animals on the sucrose diet with supplementary thiamin and flavin. These derivatives of pyridine had neither preventive nor curative action for typical symptoms of the florid dermatitis. Whereas the combination of the filtrate fraction with the alcoholic pyridine eluate produced an increased rate of growth, the addition of nicotinic acid or amide to this eluate did not, therefore, in rats, these compounds do not possess the complete properties of either the filtrate or the dermatitis factors.

A dose of 75 mg. of the rice polishings concentrate fed to depleted rats contained adequate quantities of the growth and dermatitis factors. A 25 mg. level under similar conditions resulted in a low growth rate which was not improved by the addition of nicotinic acid or amide. It appeared that this active component of the rice polishings concentrate was not identical with nicotinic acid or amide. In no case were delayed growth responses observed either when active fractions were fed to the rats or when they were maintained on the basal regime plus nicotinic acid or amide for four weeks.

Nicotinic acid amide, 1 mg., demonstrated no supplementary effect when administered together with 5 mg. of yeast adenylic or nucleic acids to animals receiving thiamin, riboflavin and the dermatitis factor (20 mg. pyridine eluate) over a 14-day period.

Summary. The extracts and eluates derived from rice polishings represented concentrated sources of the dermatitis factor as shown by curative properties and growth effects.

The filtrate from repeated adsorptions possessed properties which increase the growth-promoting action of the pyridine eluate.

[5] Frost, D. V., and Elvehjem, C. A., *J. Biol. Chem.*, 1937, **121**, 255.

† Van Veen[6] identified adenine, uracil, cytosine, and nicotinic acid in the eluates from adsorption products of rice bran, and Funk[7] isolated crystalline nicotinic acid from purified extracts of rice polishings.

[6] Van Veen, A. F., *Mededeel. Dienst. Volks. Nederl.*, 1931, **20**, 80.

[7] Funk, C., *The Vitamines*, Baltimore, Williams & Wilkins, Trans. by Dubin, H. E., from the 2nd German ed., 1922, p. 171.

In this study nicotinic acid or amide were not equivalent to the filtrate or the dermatitis factors in the nutrition of rats. Nicotinic acid or amide had no supplementary effect when combined with concentrates from rice polishings and with yeast adenylic or nucleic acid.

9627 P

Effect of the Papilloma Virus (Shope) Upon the Tar Warts of Rabbits.

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When the papilloma virus is injected into the blood stream of rabbits previously tarred on the ears for 1½ to 3 months, growths rapidly arise on the tarred skin, often in great numbers. Some are squamous cell carcinomas,[1] usually multiple, and frequently metastasing.* We have studied the phenomenon in more than 70 rabbits, with 90 tarred controls. In none of the latter has a cancer developed.

Many of the growths that follow upon injection of the virus appear where no localized proliferation was previously visible, but others derive from pre-existing tar warts, which start growing rapidly, alter in aspect, and not infrequently manifest malignancy. After tarring is stopped, most ordinary tar warts disappear and the others become indolent, whereas the virus-stimulated warts keep on enlarging. Some are now carcinomatous but may undergo conversion into characteristic virus papillomas, as a scrutiny of several hundred specimens has shown; and others have become hybrids, neither ordinary tar tumors nor ordinary virus tumors but peculiar papillomas of malignant appearance, which change to squamous cell carcinomas almost at once. Not a few of the warts, however, though greatly stimulated by the virus (as proven by weekly records of their size), retain the morphology of the tar tumors.

To learn more, opportunity was provided for the virus to infect tar warts *in vitro*. The warty tissue was punched from the ears, a

[1] Rous, Peyton, and Kidd, J. G., *Science*, 1936, **83**, 468.

* Using benzpyrene instead of tar, Lacassagne and Nyka have confirmed our findings (*Bull. de l'Assoc. Française pour l'Etude du Cancer*, 1937, **26**, 1).