

Through the help and cooperation of Doctor E. C. Dickson, Stanford Medical School, San Francisco, sections were made of the original animal lesions and also of the infected guinea pigs. The results confirmed the cultural findings. Table II is a summary of the laboratory examinations made, with the results.

It may be concluded from the above table that the lesions from 7 slaughtered animals, *i. e.*, 6 cattle and 1 sheep were proven to contain *Coccidioides immitis*. Giltner¹ encountered the infection in bovine bronchial and mediastinal lymph glands from an animal slaughtered in San Diego, California. Although very little is known of the mode of infection of *coccidioidal granuloma* it does not seem likely that humans are infected from animals; but, rather that both man and animals are infected from the same source.

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Experiments with Reference to the More Heat-stable Factor of the Vitamin B Group (Factor P-P, Vitamin B₂ or G).*

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Sparing solubility of this substance in alcohol. Extraction experiments with 80% alcohol were deemed of special interest because of the use of such alcohol extracts by Goldberger and associates¹ and because alcohol of almost the same concentration (79%) had been used by Osborne and Wakeman² for the precipitation of their "fraction II" which contained a relatively high concentration of both of the recently differentiated water-soluble factors required in the growth of rats.

In the present experiments, 400 gm. of air-dry baker's yeast were treated with 1500 cc. of alcohol (80% by weight), thoroughly stirred, and allowed to stand at room temperature (20°-25° C.) for 24 hours; then filtered with suction, and the yeast washed on a Büchner filter with 750 cc. of alcohol of the same strength; then again stirred with 1500 cc. of the alcohol, allowed to stand 24 hours,

¹ Giltner, L. T., *J. Agr. Research*, 1928, xiv, 533.

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¹ Goldberger, J., Wheeler, G. A., Lillie, R. D., and Rogers, L. M., *United States Public Health Reports*, 1926, xli, 297.

² Osborne, T. B., and Wakeman, A. J., *J. Biol. Chem.*, 1919, xi, 383.

filtered and washed as before. The residue was dried in the air at room temperature.

The extract obtained by combining the 2 filtrates and the washings was a clear yellow solution of pH 6.1. It was concentrated on the steam bath and evaporated at room temperature on corn-starch. Feeding experiments indicated that this extract contained the greater part of the antineuritic vitamin but scarcely measurable amounts of the heat-stable factor. The extracted residue, however, was somewhat less potent than the original yeast as a source of this latter factor.

Extraction as above with alcohol 60% (by weight) gave a greenish yellow-brown solution of pH 5.9, which frothed easily. Feeding experiment with the extracted residue indicated that it retained about one-half of the heat-stable factor of the dry yeast. The evaporated extract, while showing decided activity, seemed less potent than the extracted residue.

Similar extraction with alcohol of 95% (by weight) yielded a very pale solution of pH 6.4 which contained about 7% of the solids of the yeast but not enough of this heat-stable factor (vitamin B₂ or G) for detection by the method here used. The extracted residue seemed as potent as the original yeast.

The experiments here reported suggested the possibility of some destruction of the heat-stable factor, perhaps through oxidation, during the preparation and drying of the alcohol extracts.

More detailed studies of alcohol extraction and of the quantitative determination of vitamins B₁ and B₂ (the anti-neuritic and the more heat-stable factors) are in progress in this laboratory.

General plan of feeding experiments. Healthy young albino rats were placed when 28-30 days old upon a basal diet adequate in other respects but freed as completely as was here practicable from the heat-stable factor to be studied; that is, upon the Sherman and Spohn³ "vitamin B-free" basal diet (using this expression in the older sense) which had been modified by the addition of an alcoholic extract of ground whole wheat previously proven to furnish an adequate supply of the antineuritic vitamin. In this way the heat-stable substance (although possibly supplied in small amounts) became the sole growth-limiting factor, so far as present knowledge indicates.

The rats were kept upon the basal diet alone until growth had ceased and a slight loss in weight had occurred. Following this depletion period, they received the basal diet with and without quantitatively graded additions of the original dry yeast and of the various

³ Sherman, H. C., and Spohn, A. A., *J. Am. Chem. Soc.*, 1923, xlv, 2719.

extracts and residues prepared from it as indicated above. In order to free these extracts completely from alcohol and bring them into suitable form for quantitative feeding experiments, they were first concentrated on the steam bath and then evaporated upon cornstarch at room temperature. Quantitatively graded allowances of these preparations were fed three times weekly, separately from the basal diet.

Effects of limitation of intake of this vitamin upon the weight curve and condition of the experimental animal. As in the case of the vitamin B complex and of the antineuritic factor so also of the more heat-stable factor, the feeding of graded amounts, as supplements to the basal diet, resulted in a series of weight curves graded from those of the "negative controls" to those approaching a normal rate of growth. In other words, the limitation of growth or the loss of body weight was somewhat proportional, though not in simple arithmetical proportion) to the severity of the restriction of intake to this factor (vitamin B₂ or G).

In respect to this observation and those which follow, our findings confirm and extend the work of previous investigators and, we believe, add somewhat to the definiteness and precision of our knowledge of the nutritional significance of this factor.

When this factor (P-P, vitamin B₂ or G) is excluded almost (though possibly not quite) completely from a food intake adequate in other respects, in experiments with rats of an initial age of 4 weeks, growth is quickly retarded and a loss of body weight may begin within 2 weeks; but this time interval is subject to considerable variation depending upon the completeness with which the diet has been freed from the factor in question, and, we believe, also upon the previous diet of the animal. Several weeks later, soreness of the eyes appears with abnormal secretion and a tendency to sticky lids, and to frequent rubbing and occasional scratching of the lids by the animal; in many cases the fur then falls out around the eyes, leaving bald inflamed rims; usually the mouth becomes sore, first with indications of excessive salivation, then with inflammation at the corners, sometimes progressing to an inflamed or ulcerated condition of the entire lower lip; in many cases, though less regularly, soreness of the nose develops with occasional bleeding; the animal becomes weak and tends to remain in a humped posture; in the advanced stages of this deficiency disease, diarrhea is common and there may be bloody discharges, both in feces and urine. When these cases of almost complete deprivation of vitamin B₂ or G were allowed to run a fatal course, death sometimes occurred without the

development of conspicuous skin symptoms, though the fact that an abnormal condition of the skin had actually developed was indicated by extensive desquamation occurring in those instances in which life was prolonged or health restored by curative feedings.

In general our experiments indicated that in the condition resulting from almost complete deprivation of this vitamin the eyes, nose, mouth, alimentary tract, and perhaps kidneys are apt to show more pronounced abnormalities than the skin; whereas with a less complete deprivation and a somewhat longer course of the deficiency disease the abnormalities of the skin are apt to become much more prominent.

In these latter less rapidly fatal cases, the fur becomes dry and pulls out readily. Often a definite saddle-like pattern appears on the fur of the back. An examination of the area affected may reveal small dry cream-colored scales on the skin, which later may be replaced by larger yellowish crusts, appearing on the sides of the back, the shoulders, and the chest. The skin lesions usually show a tendency to symmetry; there are occasional cases of rough, red areas upon the paws and the inside of the fore legs; there may be more or less pronounced development of other symptoms, such as have just been mentioned as characteristic of the more acute cases.

On the whole, the symptoms observed by us are sufficiently consistent with those reported by Goldberger and his coworkers and by Chick and Roscoe⁴ to justify the statement that the experimental reproducibility in rats of the conditions called pellagra-like by those investigators has been abundantly confirmed. Whether or not future usage will apply the term antipellagic to it, we would emphasize the fact that this heat-stable vitamin (B_2 or G) is evidently a substance of coordinate importance with the longer-known vitamins as an essential factor in normal nutrition and that deprivation or serious shortage of this substance results in widespread injury to the body tissues. Conversely, the liberal feeding of this substance may be expected to play a significant part in inducing a better-than-average nutritive condition—a problem which is being approached from various angles in the experimental work of this laboratory.

Curative experiments. Some of the animals showing marked symptoms of the dietary deficiency here described have been cured by the feeding of materials selected as rich in vitamin B_2 or G, without other change in their diet and without any change in sanitary conditions. These curative experiments yielded such uniformly clear-cut results as to furnish strong confirmation, if such were

⁴ Chick, H., and Roscoe, M. H., *Biochem. J.*, 1927, xxi, 698.

needed, of the view that the condition in question is essentially a dietary deficiency disease in the sense in which this term is ordinarily used by students of nutrition, although, as in the case of vitamin A, the typical clinical picture may include the results of infection or infections, the incidence and development of which become prominent under the conditions of the nutritional deficiency.