

A Well-Balanced Ration for Stock Rats.

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THERE is undoubtedly no branch of biology in which so much progress has been made during the last fifteen years as in the line of nutrition, but were it not for the extensive use of the albino rat (*Mus norvegicus albinus*) in America and Europe in connection with nutrition problems much of the valuable knowledge gained so far would not have been available. At present the white rat is used almost universally in most nutrition work that is of an exact and statistical nature; a distinction it certainly deserves because the rat is such an economical animal to experiment with in view of the fact that its size makes it possible to house a large number in a relatively small space, and to provide the necessary food at a low cost. Moreover, it also has a relatively short life span, becomes sexually mature at an early age, its reproductive activities are rapid, it is omnivorous and not at all fastidious with its diet which can be absolutely controlled, and what is more, it can easily be standardized both genetically and nutritionally, a point which is of considerable importance in nutrition work.

With the object of starting a pure-bred and standardized colony, twelve (eight females and four males) Wistar Rats of the London strain were imported in 1932 from the Glaxo Research Laboratory, London. At the present writing several generations have already been raised from the original imported animals. These stock rats are hardy, active and very tame. They are housed in large cages in a room that is well lighted, well ventilated, clean and electrically heated during the cold winter months. Clean shavings are used for bedding.

In looking through the literature for a good stock ration, it was impossible to select one that was quite satisfactory, in view of the fact that recent investigations on the essentials of a good diet made it clear that the normal rations of Steenbock (1923), Waddell and Steenbock (1928), Sherman and Burtis (1928), and Bills *et al.* (1931) can all be improved upon. The stock rations of Smith and Bing (1928), Moore and co-workers (1932), Coward and collaborators (1932), and Bacharach (1933) are no doubt adequate in all respects but they are not simple and uniform enough for our purpose. In this laboratory a suitable mixture containing all the necessary ingredients is preferred to their diets which also include fresh vegetables, meat and liver, because Kon (1931) has shown that the rat is not a wise selector of different food constituents when offered separately.

The stock ration adopted in this laboratory is a modification of Waddell and Steenbocks' (1928) normal diet in so far as parts of the yellow corn and linseed oil meal have been replaced by dried beef liver, brewer's yeast and calcium carbonate. It is a dry comminuted ration with the following composition:—

	Ground yellow corn	...	68	per cent.
	Linseed oil meal	...	10	„
	Crude casein	...	5	„
	Dried brewer's yeast	...	5	„
	Alfalfa meal	...	3	„
	Butter fat	...	5	„
	Beef liver (dried at 70° C.)	...	2	„
	Bone ash *	...	1	„
	CaCO ₃	...	0.5	„
Sodium chloride	NaCl	...	0.5	„

Fresh whole milk and tap water, fed separately, *ad libitum* daily.

The above ration has been in use now for more than a year with excellent results. 98 Per cent. of the litters weighed 40 to 60 grams at an age of 23 days. All the litters were reduced to 6 on the day of parturition as practised by Macy, Outhouse, Long and Graham (1927).

Beef liver was incorporated in the ration because it has been shown in different laboratories that liver stimulates food consumption and utilization, general growth and lactation. [Osborne and Mendel (1926), Evans and Burr (1927), Mapson (1932), Seegers and Smith (1932), Graham and Griffith (1933), Seegers and Smith (1933), and Bahrs (1933).] Whether liver owes its above qualities to its high vitamin B₂ content (Cuha, 1931, Graham and Griffith, 1933) or to some yet unknown essential dietary factor remains to be seen. However, Karrer and v. Euler (1933) have shown recently that a vitamin B₂ extract from liver possessed remarkable growth-promoting power when fed to rats. Nevertheless, by the addition of 5 per cent. brewer's yeast, the above ration is also well supplied with the vitamin B complex. The addition of yeast was considered necessary in view of the fact that the results of Macy *et al.* (1927), Evans and Burr (1928), Sure, *et al.* (1929) and Clayton (1930) show that nursing rats need an abundant supply of vitamin B if successful lactation is to take place. The yeast is obtained from the Castle Brewery, Johannesburg, in a dry commercial form. It contains 7.2 per cent. nitrogen and has been found effective in aiding growth and preventing polyneuritis when added at a 5 per cent. level to a vitamin B-free ration.

The fat soluble vitamins A, D and E are also present in optimum amounts. As a matter of fact the ration is too rich in vitamin A when the young animals are to be used for the assay of this vitamin. Of course, in assaying vitamin A it is always better to regulate first the storage of this vitamin in the animals either by the method of Nelson (1928) or that of Garrett and Mitchell (1933) before they are put on the experimental rations. Vitamin C, although not exactly necessary in the diet of the rat, is supplied in the fresh whole milk.

* The bone ash was prepared by ashing commercial bone meal in an electric muffle at a bright red heat until a nice white ash was obtained. The ash was then ground to a fine powder in a large iron mortar.

The alfalfa meal, in addition to containing an apparent new growth-stimulating factor as shown by Mason (1928), also serves as a good roughage.

The crude fibre (determined by Mr. Roets), ash and protein (total N-N in yeast $\times 6.25$) contents of the ration are 4.5, 3.72 and 15.69 per cent. respectively. The nitrogen of the yeast was not calculated as protein-nitrogen in view of the fact that Still and Koch (1928) when measuring by Mitchell's method the biological values of diets containing one half of the nitrogen from yeast and one half from casein (total N = 2.9 per cent.) found little supplementary relation between the two proteins. The distribution of food energy when expressed in terms of percentages of the total energy* of the ration is: carbohydrates 68.2, fats 15.0 and protein 16.8. Approximately 65 per cent. of the protein came from plant and 35 per cent. from animal sources. It will be seen that the protein content (16.8 per cent.) of the ration falls between 14 and 18 per cent., the range in which Slonaker (1931), (1931a), (1931b), (1931c), (1931d), (1931e), obtained the best results for growth, reproduction, activity, etc., when taken as a whole. Bing and co-workers (1932) believe that the protein requirements of the mouse are certainly fulfilled by diets containing 15.6 per cent. protein (casein), and that the protein requirements of rats and mice are nearly the same.

The calcium, phosphorus and magnesium contents are 0.61, 0.44 and 0.16 per cent. respectively, with a Ca : P ratio of 1.39 which porportion has been found by Simmonds (1924), Bethke and Edgington (1927), and Bethke, Edgington and Kick (1933) to be the optimum or near optimum for the rat and for pigs. In a more recent paper by Bethke, Kick and Wilder (1932) further evidence is given that a Ca : P ration between 2.00 and 1.00 is the most favourable for growth and bone formation in the rat. The iodine content is 33 μ per 100 grams of ration as determined by Dr. Blom (1933) whose co-operation is appreciated.

SUMMARY.

A dry comminuted ration for stock rats is described which is considered to be well balanced and adequate in all respects. It contains 68.2 carbohydrates, 15.0 fats and 16.8 protein expressed in terms of percentages of the total calories yielded by the ration. Its ash, calcium and phosphorus contents are 3.72, 0.61 and 0.44 per cent. respectively with a Ca : P ratio of 1.39.

LITERATURE CITED.

- BACHARACH, A. L. (1933). Investigation into Vitamin A-free Basal Diets. *Biochem. J.*, Vol. 27, pp. 5-16.
- BAHRS, A. M. (1933). The Effect of Adding Liver to the Diet upon the Growth and Reproduction of Rats. *Amer. J. Physiol.*, Vol. 105, pp. 262-265.
- BETHKE, R. M., AND EDGINGTON, B. H. (1927). The Calcium-phosphorus Ratio in Animal Nutrition. *Proc. Amer. Soc. Animal Prod.*, p. 13.

* Total calories include those derived from yeast and alfalfa meal.

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- BETHKE, R. M., KICK, C. H. AND WILDER, W. (1932). The Effect of Calcium-phosphorus Relationship on Growth, Calcification and Blood Composition of the Rat. *J. Biol. Chem.*, Vol. 98, pp. 389-403.
- BETHKE, R. M., EDGINGTON, B. H., AND KICK, C. H. (1933). Effect of the Calcium-Phosphorus Relationship of the Ration on Growth and Bone Formation in the Pig. *J. Agric. Res.*, Vol. 47, pp. 331-338.
- BILLS, C. E., HONEYWELL, E. M., WIRICK, A. M., AND NUSSMEIER, M. (1931). A Critique of the Line Test for Vitamin D. *J. Biol. Chem.*, Vol. 90, pp. 619-636.
- BING, F. C., ADAMS, W. L., AND BOWMAN, R. O. (1932). Protein Requirements of the Albino Mouse. *J. Nutr.*, Vol. 5, pp. 571-9.
- BLOM, I. J. B. (1933). Methods for the Micro-determination of Iodine in Biological Material. *O.Poort Jnl. of Vet. Sc. and An. Ind.*, Vol. 2, No. 1, pp. 123-130.
- CLAYTON, M. M. (1933). The Comparative Value of Milk, Egg and Meat Proteins for Reproduction and Lactation in the Rat. *J. Nutr.*, Vol. 3, pp. 23-38.
- COWARD, K H., CAMBDEN, M. R., AND LEE, E. M. (1932). A Stock Diet for Rats Bred for Vitamin Tests. *Biochem. J.*, Vol. 26, pp. 679-690.
- EVANS, H. M., AND BURR, G. O. (1928). On the Amount of Vitamin B Required during Lactation. *J. Biol. Chem.*, Vol 76, pp. 263-272.
- EVANS, H. M., AND BURR, G. O. (1927). A New Dietary Deficiency with Highly Purified Diets. *Proc. Soc. Exp. Biol. and Med.*, Vol. 24, pp. 740-743.
- GARRETT, O. F., AND MITCHELL, H. H. (1933). An Application of the Paired Feeding Method to the Quantitative Estimation of the Relative Vitamin A Contents of Foods and Artificial Concentrates. *Am. J. Physiol.*, Vol. 104, pp. 608-614.
- GRAHAM, C. E., AND GRIFFITH, W. H. (1933). Growth Factors in Liver. *J. Nutr.*, Vol. 6, pp. 179-194.
- GUHA, B. C. (1931). The Sources, Stability and Chemistry of Vitamin B₂. *Biochem. J.*, Vol. 25, pp. 945-959.
- KARRER, P., AND v. EULER, H. (1933). *Arkiv. Kiwi., Mineral Geol.* 11b, Heft 16, 4 pp.; *Chem. Abst.*, Vol. 27, p. 5100.
- KON, S. K. (1931). The Self-selection of Food Constituents by the Rat. *Biochem. J.*, Vol. 25, pp. 473-481.
- MACY, I. G., OUTHOUSE, J., LONG, M. L. AND GRAHAM, A. (1927). Technique Employed in Vitamin Studies. *J. Biol. Chem.*, Vol. 73, pp. 153-174.
- MAPSON, L. W. (1932). Evidence of the Existence of a Dietary Principle Stimulating General Growth and Lactation. *Biochem. J.*, Vol. 26, pp. 970-986.
- MASON, K. E. (1928). The Effect of Purified Diets, and their Modifications on Growth and Testicular Degeneration in Male Rats. *J. Nutr.*, Vol. 1, pp. 311-334.
- MOORE, C. U., PLYMATE, H. B., ANDREW, B. J., AND WHITE, V. (1932). Statistical Comparison of Small and Large Litters of Rats on a Normal Stock Diet. *Am. J. Physiol.*, Vol. 102, pp. 566-572.
- NELSON, E. M. (1928). Regulating the Storage of Vitamin A in Animals that are to be Used for the Determination of this Vitamin. *Science*, Vol. 68, p. 212.
- OSBORNE, T. B., AND MENDEL, L. B. (1926). The Relation of the Rate of Growth to Diet. *J. Biol. Chem.*, Vol. 69, pp. 661-673.

- SEEGERS, W. H., AND SMITH, H. G. (1932). Influence of Raw and Whole Dried Liver on Food Consumption and Utilization. *Proc. Soc. Exp. Biol. and Med.*, Vol. 30, pp. 365-6.
- SEEGERS, W. H., AND SMITH, H. G. (1933). The Presence of a New Dietary Principle in Liver. *Proc. Amer. Soc. Biol. Chem.*, Vol. 8, p. 87; *J. Biol. Chem.*, Vol. 100, p. 87.
- SHERMAN, H. C., AND BURTIS, M. P. (1928). Factors Affecting the Accuracy of the Quantitative Determination of Vitamin A. *J. Biol. Chem.*, Vol. 78, pp. 671-680.
- SIMMONDS, N. (1924). Observations on Reproduction and Rearing of Young by the Rat as Influenced by Diet. *Amer. Jour. Hyg.*, Vol. 4, pp. 1-168.
- SLONAKER, J. R. The Effect of Different Per Cents. of Protein in the Diet :
 (1931) I. Growth. *Am. J. Physiol.*, Vol. 96, pp. 547-556.
 (1931A) II. Spontaneous Activity. *Ibid.*, pp. 557-561.
 (1931B) III. Intake and Expenditure of Energy. *Am. J. Physiol.*, Vol. 97, pp. 15-21.
 (1931C) IV. Reproduction. *Ibid.*, pp. 322-328.
 (1931D) V. The Offspring. *Ibid.*, pp. 573-580.
 (1931E) VI. Weight of Mothers during Gestation and Lactation. *Ibid.*, pp. 626-634.
- SMITH, A. H., AND BING, F. C. (1928). Improved Rate of Growth of Stock Albino Rats. *J. Nutr.*, Vol. 1, pp. 179-189.
- STEENBOCK, H. (1923). A Satisfactory Ration for Stock Rats. *Science*, Vol. 58, pp. 449-450.
- STILL, E. U., AND KOCH, F. C. (1928). The Biological Value of Yeast Proteins for the Rat. *Am. J. Physiol.*, Vol. 87, pp. 225-248.
- SURE, B., WALKER, D. J., AND STUART, E. H. (1929). A Quantitative Biological Method for the Study of Vitamin B Requirements of Nursing Young of the Albino Rat. *J. Nutr.*, Vol. 1, pp. 139-153.
- WADDELL, J., AND STEENBOCK, H. (1928). The Destruction of Vitamin E in a Ration Composed of Natural and Varied Foodstuffs. *J. Biol. Chem.*, Vol. 80, pp. 431-442.