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Author: Constance Kies, Ph.D.
Professor of Food and Nutrition

Address: Department of Food and Nutrition
University of Nebraska
Lincoln, Nebraska 68503, U.S.A.

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Cereal and plant products are the chief source of dietary protein for the majority of people in the world today. In general, plant proteins are considered to be of lower nutritional value than those of animal origins. However, increasing populations will probably increase rather than decrease human dependence on these resources.

Various approaches may be used in the improvement of protein value of basic foods such as wheat grain materials. Whether these changes are based on genetic production or processing alterations, there is a need to assay supposed improvements.

At the University of Nebraska, the Department of Agronomy and the Department of Food and Nutrition recently entered into an agreement to cooperatively investigate the protein value of various wheat materials. Although both departments have other independent projects concerned with nutritional evaluation of cereal materials, the objectives of this project are as follows.

1) To set up a mass testing operation for biological evaluation of various wheat materials as sources of protein.
2) To attempt to isolate and define causes of variability among different wheat samples in relation to protein value.
3) To lay a foundation for establishment of general guidelines for predicting the value of wheat materials as sources of protein.

This paper will attempt to do two things:

1) Review our philosophy for what we are doing and why we have selected the procedures we have.
2) Review our progress. This project really is only fairly beyond the limitation stage.

One of the first problems to be faced in the evaluation of cereal products such as those based on wheat is that of goals. Suitable assay procedures should be carefully matched with ultimate goals. Unlike many cereals, wheat is primarily

consumed directly by humans. Hence, goals for improvement in wheat protein should be matched by goals for human nutrition. Expectations for human nutrition in the large sense, have not been fully defined. Most practical assay tools for judging adequacy of protein were originally derived for use in animal nutrition where greatest growth for least feed expenditure was the goal. Is to have bigger humans the goal of human nutrition? Is it more truly to have longer life span or to improve "quality" of life? If these correlate well with growth, no problem exists; but this has not been shown to be the case. Practical evaluation tools are difficult to develop with well-defined goals for human nutrition as a whole. Even so, efforts must be made for carrying out evaluation procedures even while recognizing their limitations.

Various interacting factors are involved in determining the protein value of wheats as sources of protein. Some of the defined direct determinants are thought to be as follows:

1) Amino acid proportionality patterns. Lysine content has received much stress in the case of wheat but other interrelationships may well be involved.
2) Total protein content.
3) Protein digestibility and amino acid availability.
4) Total nutritional environment.

Indirect determinants of value of wheat materials as sources of protein may involve the following:

1) Palatability and acceptability. Regardless of the nutritional merits of a product if people won't eat it or if dietary habits limit its inclusion in the diet to very small amounts, its value is nil.
2) Availability
3) Cost

4) Other constituents of the wheat materials. These may enhance or detract from value as protein resources.

5) Other constituents of the diet. For example, if the diet contains legumes, usually fairly good sources of lysine, the relatively low lysine content of many wheat lines may be unimportant.

Obviously, no single assay procedure could possibly take all of these factors into account. An idealized approach to evaluating the value of a supplementary protein would involve a sequential progression from chemical evaluations, to biological evaluations with animals, to biological evaluation with humans, to controlled field and uncontrolled field trials. For routine evaluation of large numbers of test materials such a procedure is prohibitive in terms of time, money and limitations of test materials. In the Nebraska project, the first stage involving chemical evaluation of amino acid proportionality patterns (particularly lysine) and total protein content of the wheat materials is being carried out in the laboratory of Professor Paul Mattern, Department of Agronomy. The second stage evaluation involving small animal biological evaluation is being completed in our laboratories. The mouse was selected tentatively as the animal model for biological assay in our laboratory for the following reasons:

1) Low ration requirements of this animal makes practical the biological evaluation of materials which, in some cases, are available only in small amounts.

2) Relatively large amount of information available on nutritional requirements of the animal making data derived of more valid use in terms of transfer of conclusions to other species including man.

3) Uniformity of animals due to long inbreeding reducing number of test animals needed per group feeding evaluation.

4) Availability of weanling mice from commercial colonies eliminating the need for establishing (at this time) of an independent breeding colony which greatly simplifies the entire undertaking.

5) Acceptability by the scientific community of this animal as a suitable bioassay tool.

Throughout our studies weanling mice (18 g weight at start of test runs) of the Swiss Webster strain have been used.

Several methods of biological assay are available. The commonly used PER method (protein efficiency ratio method) was considered. This method involves feeding rations containing equal levels of protein (traditionally 10%) to growing animals for test period of usually 28 days. PER is equal to the weight gain of the animal per unit protein consumed. This method is of value for answering questions concerning protein quality. Results obtained reflect adequacy of amino acid proportionality patterns and protein digestibility primarily.

Humans eat in terms of portions or measures of food rather than in terms of portions or measures of nutrients. For example, people select slices of bread; they do not make selections on the basis of grams of wheat protein. Thus, among wheat-eating peoples if other variables remain unchanged, an increase in protein content of wheat will result in an increase in total dietary protein proportional to the amount of wheat in the diet. People will not reduce the amount of wheat consumed on purpose to maintain a steady-state protein intake. This being the case, we decided to do the mouse biological assays of the wheat samples using rations formulated to contain equal percentages of wheat grain or wheat products rather than equal percentages of wheat protein. Thus, the rations vary in protein content proportional to the protein content of the test product. Ration consumed per unit of weight gain is measured. This method is referred to as FER (feed efficiency ratio). An increase in FER number value is indicative of a decrease

in the value of the wheat as a source of protein. Results of this method are dependent in theory upon protein quality (amino acid proportionality pattern), protein quantity, and digestibility/availability.

The initial studies were devoted to an attempt to determine a desirable ration formula. For testing purposes an ideal formula should not give maximal growth of animals but should allow for separation among animals fed different rations. Thus, the range in likely protein contents as incorporated into the ration must be high enough to allow some growth but not so large as to allow maximal growth for most samples tested. Neither no growth or maximal growth allows for separation among samples, the objective of the project. The ration formula which works for us in bringing samples into testing range is as follows:

Wheat	75%
Sucrose	9.8%
Corn oil	10%
Mineral mix	5%
Vitamin mix	0.2%

A total of 600 g of grain or less is needed for each 5 mouse, 28 day, assay.

Biological assay evaluation demands the use of control materials against which all samples are measured in terms of animal performance. Casein has been most commonly used for this purpose. For sake of tradition, we, too, use casein control data but to increase the relevancy of the data, standard wheat materials are also used as controls. After all, wheat is more likely to replace wheat rather than wheat replacing casein in practice. We have been able to get good reproducibility in FER values for control materials on various runs.

Reports of value of various lines of wheat grown at various locations as sources of protein is somewhat tedious in a report such as this. Thus far we

have collected insufficient data for general conclusions to be made. However I thought that you might be interested in spread of data and trends.

On Figure one, mean growth of mice fed 30 wheat materials is plotted against amount of ration consumed. Each dot represents the mean of 5 animals fed each wheat material for 28 days. The encircled dots are casein control values. Growth is plotted on the vertical axis and ration consumed on the horizontal axis. Mean growth of animals fed the various materials ranged from 5.3 g to 17.6 g over the 28 day period. Mean ration consumed ranged from 87 to 124.5 g. As one might expect there is seemingly a tendency for greater growth with increased feed consumption. However, this relationship is not as clear-cut as one might expect. Biological evaluation would be much easier if one simply used growth as an evaluation. The work of measuring feed consumption is time-consuming. However the variation in ration consumption illustrated here suggests the necessity of taking this measurement into account. FER value obtained by dividing weight gain by ration consumed in part eliminates this factor. However, this is not completely the case because efficiency of ration for promoting growth is not the same at all points on the growth scale.

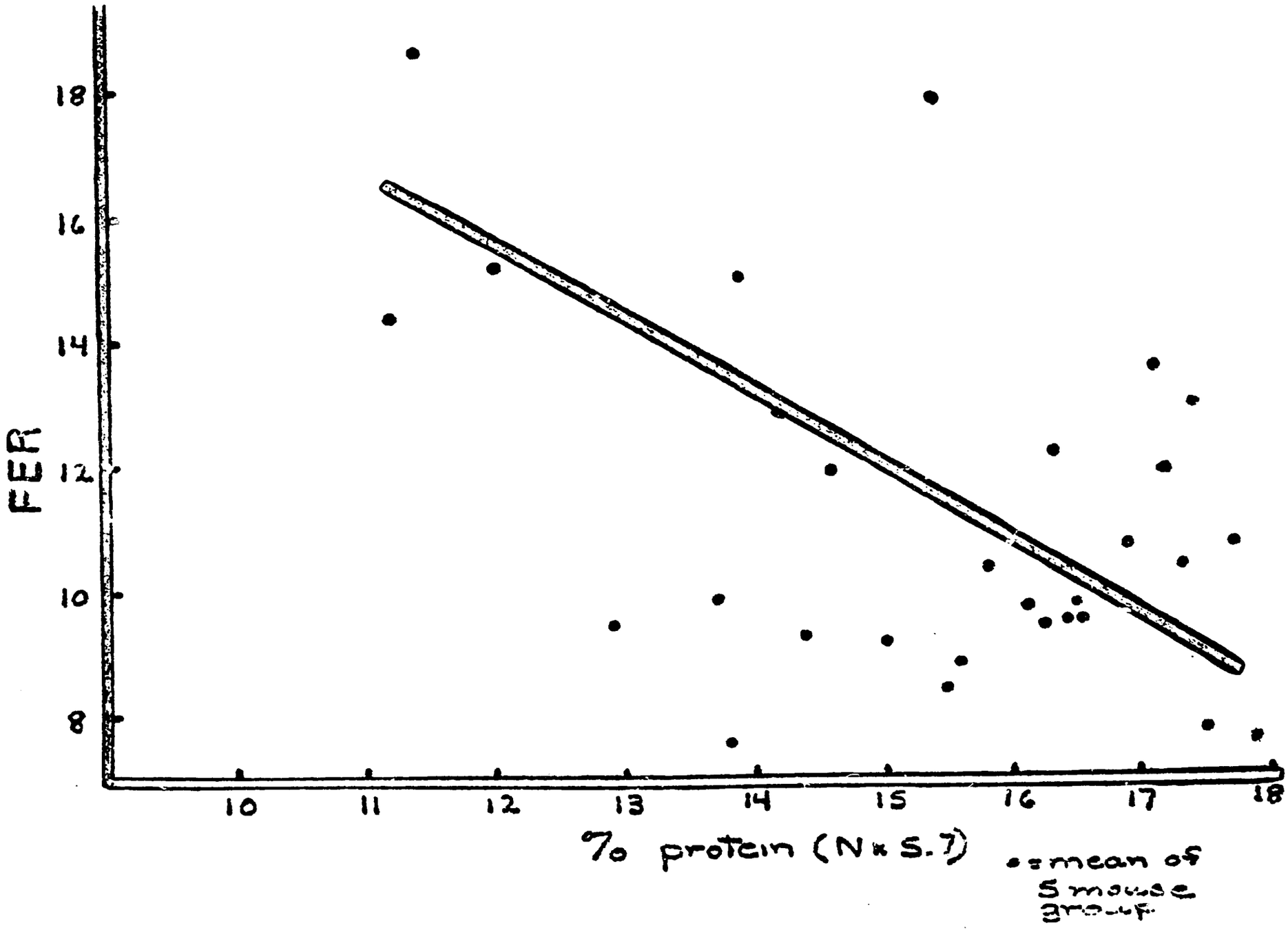
Figure 2 compares FER values and crude protein levels in the test materials. FER values are charted on the vertical axis and protein content on the horizontal axis. Each dot represents the mean value of 5 mice fed each wheat material. FER values ranged from 6.3 to 21.5 while protein content of the test materials ranged from 10.75 to 17.88%. An increase in FER value denotes a decrease in nutritive value of the ration. Seemingly the value of the wheat material as a source of protein tends to be related to the protein content of the grain. This is particularly true at the extremes of protein content. However, in the middle range, great variability exists. This may be related to variations in digestibility or lysine content. These factors are under investigation. Other factors may also be involved.

An important spin-off project from this testing program, my co-researcher Dr. Hazel Fox and I believe, is a study attempting to determine how will the results of biological evaluation of wheat materials using mice as bio-assay tools correlate with biological evaluation with humans. After all, this type of project is worthless if the results do not closely approximate results in the specie for which the materials are actually intended.

Three test wheat materials were selected: Scout 66, Scoutland, and Gage. Skim milk and casein were selected for positive controls. House bioassays were more extensive than usual because of relatively unlimited amounts of available material. These evaluations included rations formulated at equal quantities of grain (75% grain) fed at two levels of vitamin supplementation and rations formulated at equal levels of grain protein (7% and 10%).

Human bioassays included tests with adults and growing adolescent boys. In laboratory controlled study with humans procedures are somewhat different than with animals. Instead of formulating rations according to formula and allowing free consumption, in human studies it is pre-decided how much of the test material each subject will consume each day. Instead of feeding uncooked ground flour, it is necessary to incorporate the test materials into at least a semi-palatability product. In this study baked, yeast risen rolls were used. Evaluations were carried out at equal intakes of protein (4 g N per day) at two levels of vitamin intake and at equal intakes of grain (150 g per subject per day). The study with adolescent boys involved comparisons at equal levels of protein intake (4 g N per day). In human studies, the nitrogen balance technique is used as the chief means of protein evaluation. The feeding phases of these studies have been completed, however, evaluation of data is still in process. By cooperation with other laboratories we hope to see the expansion of this comparative data to other animal species.

Solution of world nutrition problems requires many approaches by many disciplines. I am delighted to be involved with the area of agronomy in their approach to improvement of human nutrition through improvement in wheat quality. I believe that only through cross-disciplinary cooperation can solutions to the multi-causal problems with which we are faced be solved.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18
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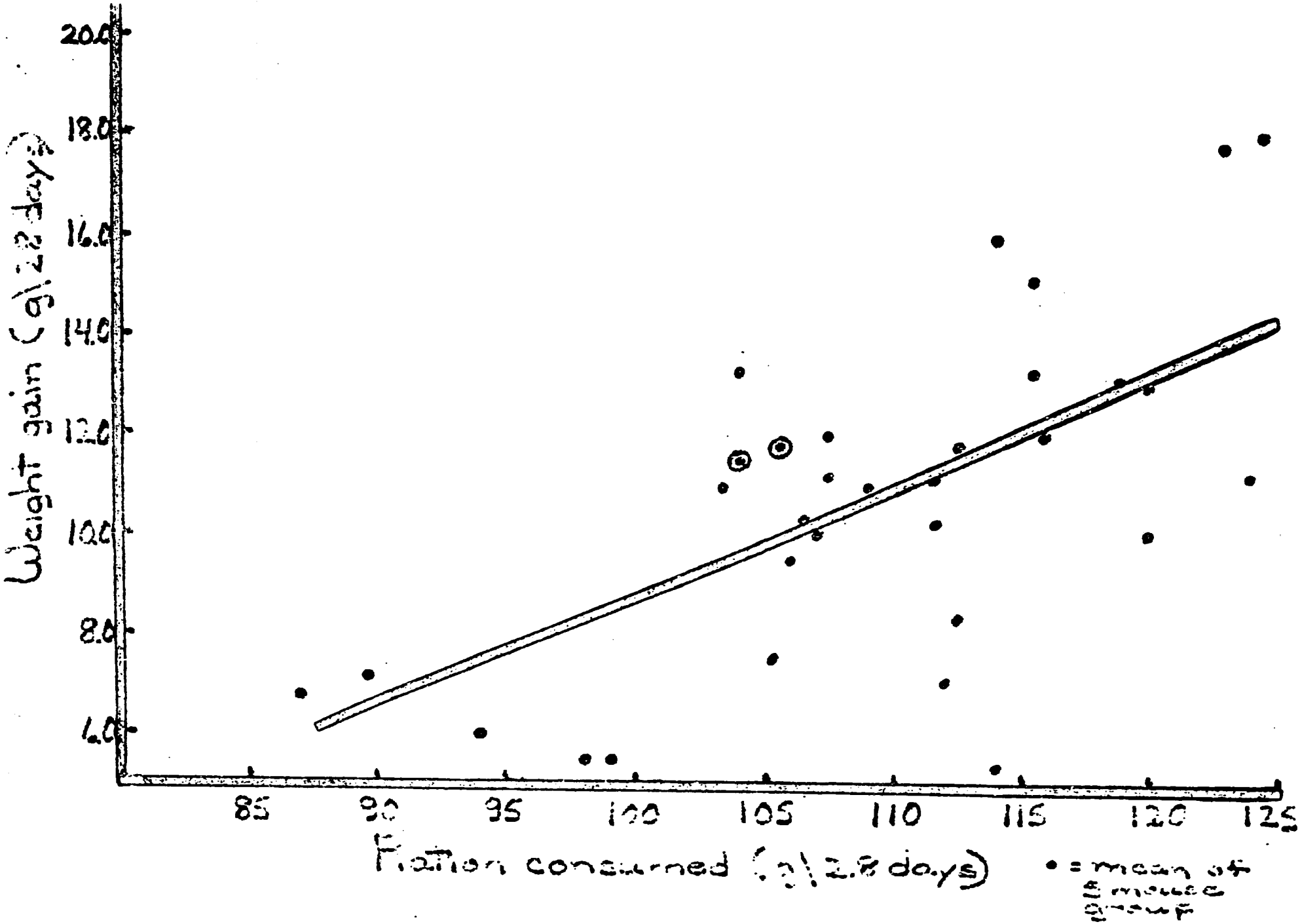
% protein (N x 5.7)
• = mean of 5 mouse group


Weight gain (g)/28 days
20.0
18.0
16.0
14.0
12.0
10.0
8.0
4.0
85
90
95
100
105
110
115
120
125
Ration consumed (g)/28 days
• = mean of 5 mice group