AN IMPROVED PROCEDURE FOR METABOLISM EXPERIMENTS.

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INTRODUCTION.

There are two general types of metabolism experiments: those of relatively short duration covering several periods, each from a few days to a week in length; and those carried out over as long a period as possible. As examples of the first type we have such investigations as the study of the effect of a given substance upon the nitrogenous equilibrium, or the study of the ability of the animal body to divert urea or other excretory nitrogen for the synthesis of special conjugate radicles of interest in intermediary metabolism; other examples will doubtless suggest themselves to the reader. As illustrations of metabolism experiments extending over months rather than days may be cited the investigations of Abderhalden and his collaborators into the ability of completely digested protein to maintain nitrogenous equilibrium over long periods and to replace adequately the intact protein molecule in nutrition. Some of Abderhalden’s experiments lasted over a period of 74 days.

Examination of the protocols presented in Abderhalden’s paper (1912) discloses the fact that loss of the desire to eat on the part of the experimental animals presented quite an obstacle to the complete success of the experiments. This failure of appetite appeared anywhere from the 7th to the 21st day of the experiment, a fact that is striking in its similarity to the behavior of our own animals which were fed diets adequate in all respects except vitamin B (and vitamin C?). Examination of Abderhalden’s diets makes it

So far as the writer is aware, no one has demonstrated that the dog requires vitamin C; the experience of this laboratory certainly has not
appear highly probable that his animals were losing their appetites as a result of a lack of vitamin B. Other instances where loss of appetite interfered with the successful prosecution of research in metabolism experiments might be cited; it is possible that in many of these cases, failures, that were attributed to monotony of diet and other seemingly more obvious causes, were really due to an absence of vitamin B from the diets being fed. Therefore, any procedure which serves to prevent loss of the desire to eat in experimental animals seems worthy of consideration and description.

Karr (1920) observed a relation between vitamin B and the appetite in the dog; the writer's investigations (1921) have confirmed and extended his findings. Such a relationship is of peculiar interest to students of metabolism and, in view of the author's most recent experiments, may be made to render a valuable service by being used to make experimental animals eat during the periods of metabolism experiments.

In his experiments Karr used as sources of vitamin B, dried brewery yeast, dried bakery yeast, a suspension made by squeezing canned tomatoes through cheese-cloth, and a crude concentrated preparation of this vitamin made from yeast according to the directions of Osborne and Wakeman (1919). In addition to confirming his results with dried brewery yeast and tomato juice, the present writer found that alcoholic extracts of rice polishings, wheat embryo, and navy bean—all of which were demonstrated by tests on pigeons to contain vitamin B—were likewise effective in restoring the appetite to dogs which had been subsisting on a diet lacking this factor.

While these experiments were being carried on, a commercial vitamin B concentrate, prepared from yeast in accordance with the main directions of Osborne and Wakeman, became available, this material being prepared by Dr. I. F. Harris and marketed under the name "Yeast Vitamine Powder (Harris)." Tests of this product for its content of vitamin B, as carried out in this laboratory on young rats by the Osborne and Mendel technique, demonstrated this, some of our animals having been fed on a diet lacking this accessory for over 200 days without the appearance of any symptoms of scurvy whatever.

*Sold by the Harris Laboratories, Tuckahoe, New York.
strated it to be a highly concentrated form of vitamin B; it was therefore tested for its appetite-promoting power on dogs by the same methods that had been employed in the earlier experiments with the other products containing the water-soluble vitamin. Such tests gave positive results. Trials with different doses of this material showed a quantitative relationship to exist between the amount of vitamin B administered by means of this product, the size of the animal in terms of the body weight, and the number of days over which the appetite was restored. The details of these experiments will appear in extenso elsewhere.

The minimum amount of "Yeast Vitamine Powder (Harris)" required for the maintenance of appetite in a dog fed in accordance with the general conditions set up in my experiments is approximately 40 mg. per kilo of body weight per day. A more definite statement in this regard does not appear to be warranted in view of the amount of error encountered in weighing the hygroscopic powder and transferring it to a gelatin capsule, the slight differences in vitamin B requirement which may be associated with age, or sex, the possible differences of vitamin B content in various samples of the commercial product, and certain other more or less poorly defined factors which may exert an influence on the relation, but which cannot easily be controlled—the oestral cycle for example—or which may not be discoverable until more exact methods of research in this field are possible. A very safe minimum dose of this product for students of metabolism to use, in view of the above considerations would appear to be 60 mg. per kilo of body weight per day. Tests to determine the minimum dose of a wheat embryo preparation containing vitamin B and available in large quantities are now being made following the same method employed in ascertaining the minimum dose of this yeast product.

The new procedure for metabolism experiments which the writer has to suggest utilizes his determination of the minimum dose for dogs of vitamin B in terms of the "Yeast Vitamine Powder (Harris)," when the animal is fed under certain definite conditions, together with other observations which have been made in the course of his investigations.

3 Personal communication from Dr. A. H. Smith.
The New Procedure for Metabolism Experiments.

The first important principle upon which the new procedure is based is that the diet for the dog shall consist of a mixture of isolated food substances which, when fed, will supply the body with everything that is necessary for proper nutrition (except vitamin B), or which lacks only one thing (other than vitamin B); namely, the variable under consideration. The second important principle followed is that the vitamin B shall be administered apart from the food mixture above mentioned. It is appreciated that the feeding of a purely "synthetic" food mixture in metabolism experiments using dogs as experimental animals is a procedure for the successful accomplishment of which students of metabolism have striven, and the writer makes no claim to originality for this general idea. However, no one else, except Karr who initiated the work which the author has extended, appears to have made an extensive study of the conditions under which artificial mixtures of foodstuffs may be fed successfully to dogs over long periods.

The Diet.

A synthetic diet, adequate in all respects as far as this is possible, and suited for the particular problem being investigated, is devised, great care being taken to make it as perfect as possible with respect to its content of (1) protein of excellent quality,4 (2) vitamin A,5 (3) roughage, and (4) a suitable salt mixture. Vitamin B—and this is a very important feature of this new procedure—is supplied daily by the administration apart from the rest of the food of a gelatin capsule containing the requisite amount of Yeast Vitamine Powder (Harris); i.e., 60 mg. per kilo of body weight per day.

Calculation of the Diet.—The writer's practise has been to construct the diet on a kilo of body weight basis, or to form what might be called a kilo unit of food. It has been his experience that dogs weighing 7 kilos and over require for the maintenance of

4This, of course, would not be done if the plan of the experiment demanded the elimination of all protein nitrogen from the diet.

5Steenbock, Nelson, and Hart have shown the dog to be sensitive to the absence of this vitamin from the diet (Steenbock, H., Nelson, E. M., and Hart, F. R., Am. J. Physiol., 1921-22, lviii, 14).
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body weight between 70 and 80 calories per kilo per day. Following the practise of Karr—one which has been justified by the author's experience—80 calories has been selected as the number to be desired in the kilo unit of food. Dogs weighing considerably less than 7 kilos require slightly more energy intake in order to maintain their body weight; this fact, however, does not invalidate the choice of 80 calories as a unit quantity. Any variation in energy intake from this figure due to the size of the animal will not seriously affect the intake of nitrogen and salt mixture if liberal quantities such as 0.8 gm. of the former and at least 0.2 gm. of the latter be furnished with every 80 calories or kilo unit of food.

Each kilo unit of food thus prepared furnishes (1) 80 calories, (2) 0.8 gm. of protein nitrogen, (3) from 32 to 36 calories in the form of fat, this foodstuff in grams constituting not over 25 per cent of the whole, (4) 0.4 gm. of agar or of bone ash, (5) 0.2 gm. of salt mixture, and (6) either 7 per cent of butter fat or 2 per cent of cod liver oil as a source of vitamin A. An example of such a diet is given in Table I.

At the risk of appearing to elucidate in detail what may seem quite obvious, the following suggestions are presented for calculating the kilo unit of food. They may serve to make easy for others what has been learned by more or less laborious calculation by the author.

First, determine the amount of the protein required to furnish 0.8 gm. or the amount of nitrogen decided upon, using for this purpose the figure for nitrogen content obtained upon analysis of the material. Correct for the non-protein impurities of the sample and calculate the approximate number of calories yielded by the amount of pure protein present in the preparation which furnishes the desired quota of nitrogen. Allot from 32 to 36 calories to fat, divide the particular number selected by 9, and allot the resulting figure in grams tentatively to fat. With sucrose supply the remaining calories in an amount sufficient to make a total of 80. Now

*The reason for feeding so much nitrogen with every 80 calories has been indicated. The amount of nitrogen to be given may very properly be less than this figure; however, if it is less, due consideration should be given to the amino-acid make-up of the protein in order that one or more amino-acids may not thus be made limiting factors in the diet and, therefore, factors influencing the results of the experiments. Should the metabolism of such a condition, i.e. where the low content of an amino-acid is a limiting factor in the diet, be the object of study, one method of approach of course would be to feed a suitable protein on a low plane of nitrogen intake.*
allot 0.4 gm. to agar (or bone ash) and 0.3 gm. to salt mixture. Add the several items and thus determine the total weight in grams of the kilo unit of food. Translate all amounts to a percentage basis. If the fat proves to be high—much over 25 per cent—diminish it, making up the calories by an increased amount of sucrose. This final adjustment of fat and sucrose may prove the most laborious of all the steps in the calculation; in diminishing fat and increasing carbohydrate it is necessary to determine a new total weight of the kilo unit and to recalculate the percentages. Finally, divide the fat so that butter fat or cod liver oil form 7 or 2 per cent, respectively, of the entire food mixture.

**TABLE I.**

**Kilo Unit of Casein Food.**

<table>
<thead>
<tr>
<th>Amount</th>
<th>Calories</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>cm.</td>
<td>per cent</td>
<td></td>
</tr>
<tr>
<td>Casein { 12.7 per cent N } { 81.9 per cent pure } }</td>
<td>6.3</td>
<td>20.8</td>
</tr>
<tr>
<td>Sucrose</td>
<td>5.84</td>
<td>23.4</td>
</tr>
<tr>
<td>Lard</td>
<td>2.88</td>
<td>25.5</td>
</tr>
<tr>
<td>Butter fat</td>
<td>1.17</td>
<td>10.5</td>
</tr>
<tr>
<td>Bone ash</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>Salt mixture*</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>16.74</td>
<td>80.2</td>
</tr>
</tbody>
</table>

*This kilo unit contains 80 calories, 45 per cent of which are furnished by fat, and 0.8 gm. of N.

†See Table II.

Calories are calculated on the following basis:

1 gm. of protein yielding 4 calories.
1 " " carbohydrate " 4 "
1 " " fat " 9 "

Sources of Materials for the Diet.—Protein.—For metabolism experiments, where protein good in quality and sufficient in quantity is desired, casein is to be recommended. It may be obtained commercially in large quantities and has a granular texture which is almost ideal for use in making the synthetic food mixture. For most purposes, perhaps, it would not require purification and a determination of its impurities. However, in view of its low content of cystine, it would seem best to supply this protein in fairly large amounts; *i.e.*, so as to furnish 0.8 gm. of

*See foot-notes to Tables II and III.
nitrogen per kilo unit of food. For experiments in which it is desired merely to secure an approximately constant daily output of urinary nitrogen, casein should prove eminently satisfactory provided its low cystine content is not allowed to become a factor influencing the results.

Other sources of protein, which have been tried and found to be satisfactory, are extracted meat and coagulated commercial egg albumin. The author has tested both of these products for their content of vitamin B, using the Osborne and Mendel rat technique, and found them to be free from any appreciable amounts of this dietary essential. Since Bateman (1916) has shown that the ingestion of uncoagulated egg white in moderate amounts by the dog results in a diarrhea, the commercial egg albumin must be coagulated before being used in the food mixture. This protein does not appear to possess any advantages over casein, when used as part of the mixture to be fed to dogs; furthermore, it is more expensive. Unless the amino-acid make-up of the egg albumin in contrast to that of casein should be one of the factors under consideration in the experiment, the writer would prefer to use casein.

There is no apparent reason why any pure protein that is well utilized and not objectionable for peculiar reasons—as is gelatin, for example—could not be used in metabolism experiments, if

**TABLE II.**

<table>
<thead>
<tr>
<th>Salt Mixture (Karr, 1920).*</th>
<th>gm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium chloride</td>
<td>10</td>
</tr>
<tr>
<td>Calcium lactate</td>
<td>4</td>
</tr>
<tr>
<td>Magnesium citrate</td>
<td>4</td>
</tr>
<tr>
<td>Ferric citrate</td>
<td>1</td>
</tr>
<tr>
<td>Iodine in potassium iodine</td>
<td>a few drops</td>
</tr>
</tbody>
</table>

*This salt mixture, when fed along with bone ash on the basis of 0.2 gm. and 0.4 gm., respectively, per kilo unit of food, the latter serving as a source of phosphate, has given successful results with the dog during periods lasting over 5 months.

*From the Valentine Meat Juice Company, Richmond, Virginia.

*This may be secured from any wholesale chemical house, or from concerns supplying the baking trade, such as the Baker's Supply Company.
the diets are constructed following the general plan presented in this paper.

Carbohydrates.—Perusal of the literature shows glucose, sucrose, maltose, dextrin, and starches to have been used as the carbohydrates in diets fed to dogs. Of these, in the opinion of the writer, sucrose is the most desirable. As a commercial article it is unusually pure, and it is comparatively inexpensive. Furthermore, it is granular in character in contrast to the other available carbohydrates, particularly starch and dextrin, which are used a great deal in the diets fed to rats. This physical property of sucrose is of advantage in that it gives the complete food mixture a granular rather than a powdery texture. Starch and dextrin are undesirable: they have the physical properties of fine flour and with these polysaccharides it is not easy to prepare a food mixture that possesses a texture pleasing to most dogs. A food that is powdery in character tends to become sticky when moistened with saliva; dogs, when eating such a food, behave much as do animals fed a mixture containing moderate amounts of granulated gelatin.

Fats and Sources of Vitamin A.—There does not appear to be any reason why lard should be supplanted in the dietary as a source of calories and as an agent with which to bind the various dry substances being used. Butter fat is to be preferred to cod liver oil as a source of vitamin A, inasmuch as experience has shown that some dogs do not like the cod-liver oil; furthermore, the efficiency of the total fat as a binder is lessened when a liquid fat is used.

Roughage.—Abderhalden tried both bone-black and bone ash in his experiments and apparently favored the latter although pointing out that not all of his animals behaved alike as regards the type of stools eliminated when the same indigestible residue was being ingested. The efficiency of bone ash as a roughage was early emphasized by Gies (1904). In his communication recommending the use of this material Gies states that no experiments were made to determine whether any of the mineral constituents of the ash were absorbed. The experience of our laboratory has been that bone ash under most conditions is very satisfactory as a roughage, as far as the production of a dry stool is concerned, when used to the extent of 0.4 gm. per kilo unit of food; however, the use of this ash is attended by a higher level of urinary phosphate.
indicating that the bone ash does not function entirely as an indigestible residue. Inasmuch as the absorption of earthy phosphates in the intestine is influenced by many factors, chief of which are those which act by precipitating the calcium salts, the actual amount of the ash phosphate that is absorbed may be quite variable. In many metabolism experiments the amount of phosphate absorbed in the intestine or eliminated by the kidney is of no significance, and in such cases bone ash is well suited for use as roughage. On the other hand, should it be desirable to avoid the ingestion of phosphate, agar may be employed to give bulk and proper texture to the feces, the amount of this polysaccharide to be used being the same as that of the bone ash; i.e., 0.4 gm. per kilo unit of food.

Mineral Constituents. —The salt mixture used in the writer's experiments is the same as that employed by Karr (Table II); whether it is the most ideal in composition when used under a variety of conditions remains to be determined by definite experiment. This mixture contains no phosphate, but when used in conjunction with bone ash has given complete success with dogs in feeding experiments lasting over 5 months. It is possible that this particular mixture would not be ideal when used along with agar-agar instead of bone ash, since the latter may contribute mineral materials to supplement those supplied by this salt mixture.

The author has devised a new salt mixture (Table III), utilizing the principle of supplying in the food daily an amount of each of the various inorganic substances equal to or slightly in excess of that eliminated through the kidney by a normal organism during 24 hours, these amounts being expressed in terms of grams eliminated per kilo of body weight per day; at the same time this mixture offers to the body certain constituents in fairly normal ratios to others—sodium to potassium, for example. 0.3 gm. of this mixture per kilo unit of food contains a liberal amount of the various inorganic substances desired in the synthetic diet.

From a theoretical standpoint any salt mixture of proven worth such as those that McCollum and his collaborators (1915) have used, or that of Osborne and Mendel (1913), could be employed as part of the kilo unit of food; the amount that should be used, considering the experiments of McCollum and other students of
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nutrition, would seem to be about 1.5 per cent, a figure which at any rate should be the minimum.

Vitamin B.—Theoretically, any concentrated form of this vitamin could be used. A product which is free from protein and which contains only relatively small amounts of any of the other foodstuffs is the most desirable for metabolism experiments. Such a product should be standardized for such use in order to reduce to a minimum the amount of material of unknown composition that is given along with the vitamin, as well as to insure the administration of a sufficient amount of the dietary essential to maintain the appetite. As indicated earlier in this paper, the "Yeast

| TABLE III. |
| Salt Mixture.* |
| Per kilo. | Percentage. |
| gm. | per cent |
| Sodium chloride | 0.1050 | 38.0 |
| Magnesium citrate | 0.0005 | 32.5 |
| KH₂PO₄ | 0.0336 | 12.2 |
| CaHPO₄₂H₂O | 0.0215 | 7.8 |
| Potassium chloride | 0.0192 | 7.0 |
| Ferric citrate | 0.0050 | 1.8 |
| Potassium iodide | 0.0013 | 0.5 |
| **Total** | **0.2761** | **99.8** |

*Where 0.4 gm. of agar-agar per kilo unit is used as a source of roughage, this salt mixture may be used on the basis of 0.3 gm. per kilo unit of food.

Vitamine Powder (Harris)" has been standardized for this purpose by the writer's experiments, and the amount to be used has been stated. Inasmuch as this preparation contains approximately 8.33 per cent of nitrogen none of which is from protein, and a No. 00 gelatin capsule contains 20 mg. of nitrogen on an average, the animal would receive approximately 3 and 5 mg. of nitrogen from the gelatin and the Harris vitamine, respectively, per kilo of body weight per day, when vitamin B is administered by this method. This amount of nitrogen is very small; certainly that derived from protein is almost negligible.

General Remarks Concerning the Diet.—Karr used water as one constituent of his dietaries. After some experimenting the
writer decided to build up his kilo unit of food without the use of water, and this has been a decided improvement. When this change is made, the large amount of food prepared at one time does not develop an odor due to rancid fat; neither does it dry out. Ordinary butter, which contains considerable water and salt in inconstant amounts, should be replaced by butter fat for this reason.

DISCUSSION.

Caspari and Zuntz (1911), in discussing how to feed animals used in metabolism experiments, pay particular attention to the problem of maintaining the appetite and suggest the use of meat extract, bouillon, etc., as aids in surmounting this difficulty. Because dogs are carnivorous, meat is advised as the basis upon which to construct the diet and sugar is suggested as of value in improving the taste. The writer's experiments (1922) have clearly demonstrated that meat extract neither restores nor maintains the appetite in dogs which have lost the desire to eat after subsisting upon a diet adequate except with respect to vitamin B. Furthermore, the use of meat in feeding dogs is quite unnecessary: purely artificial mixtures of isolated food substances, an illustration of which has already been given in Table I, may be fed with complete success over long periods. One dog in this laboratory was fed a casein diet in accordance with the method described in this paper with no failure of appetite whatever over a period of 150 days—approximately 5 months. Five other animals have been fed synthetic diets for 3 months and over with complete success.

These results demonstrate that monotony of diet is not per se the cause of failure of appetite; loss of the desire to eat is rather an expression either (1) of the failure of the food being fed to nourish the animal properly, or (2) of an adjustment of the dog to its energy requirement when offered more than is necessary of a food mixture that is adequate in all respects. Rubner says that many

10This animal was being used by Dr. H. J. Deuel, Jr., in a study of the metabolism of pyrimidines.

years of experience with dogs leads him to believe that appetite and capacity for digestion and absorption depend on the dog's requirement for energy in his given state of nutrition. A diet which a dog will greedily devour when in a room at a temperature of 0°, he will in part refuse when at a temperature of 33°. The writer has secured evidence supporting Rubner's general contention and has therefore adopted, as a control of the energy factor influencing loss of appetite, the plan of feeding only enough calories to permit maintenance in adult animals or sufficient to allow a slight increase in body weight in growing dogs. This procedure has proved successful in all cases: its importance should not be overlooked by those who wish to make their experimental animals eat voluntarily every bit of food offered during the course of metabolism experiments.

When a dog has lost its appetite due to a lack of vitamin B, the desire to eat may be restored within 1 or 2 days by giving to the animal a single large dose of this missing dietary essential; where daily doses, each containing little more than the minimum daily requirement of the vitamin are administered, several days of irregular food intake may elapse before the dog will consume immediately all the food offered. This fact should be considered when interpreting the results obtained by supplying the vitamin to any given animal.

A few words of caution should be given here regarding certain false ideas which the demonstrated relationship between appetite and vitamin B may seem to suggest. It does not follow from the work that has been done along this line, that the administration of vitamin B will restore the appetite to all animals which have lost the desire to eat. Various pathological conditions other than vitamin B deficiency may bring about a loss of appetite; in such cases it does not seem reasonable to suppose that the administration of this vitamin will restore the desire to eat; whether it will do so, however, can only be determined by definite experiments planned to answer this question.

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