AN IDEAL PREPARATION OF ANIMALS
(ON SINGLE-FOOD-CHOICE DIET)
FOR DISSECTION OF NERVES AND GLANDS
AND FOR BONE GROWTH STUDIES*

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In 1950 a brief account was given of an ideal preparation for dissection, particularly of spinal, peripheral, and autonomic nerves in rats.¹

This present report gives a revised and more complete description of the most effective so-called "single-food-choice" diet used in producing this preparation and reasons for selecting this diet. It also gives examples of uses to which this preparation has been and may be put for dissecting nerves, glands, muscle spindles, blood vessels, and lymphatics, and in studying bone growth.

Single-food-choice diets were originally devised for selecting a representative carbohydrate, fat, and protein for use in our full self-selection diet.² We had found that rats will make beneficial selections from various purified mineral solutions, so we decided to determine whether they could make beneficial selections when all ingredients of their diet are offered in purified form—a carbohydrate, a fat, a protein, and solutions of various minerals and vitamins—all offered in separate containers. To help us decide which carbohydrate, fat, or protein to use, we ran pilot experiments in which we restricted the diet of rats to a single foodstuff. All the common carbohydrates, fats, and proteins were tested in this way. We decided to use the representative of each foodstuff on which rats of a standard age and weight lived longest.

In its simplest form this single-food-choice method was later used to determine nutritive values of all the common carbohydrates, fats, and proteins, on the assumption that rats will eat as much of a foodstuff as they are able to utilize.³,⁴ Correctness of this assumption was borne out by many later experiments. The chart in Figure 1 summarizes the results of experiments on nine carbohydrates. These rats were kept in separate activity cages containing a nonspillable food cup, a graduated inverted water bottle, and a revolving drum. Bottoms of the cages were made of half-inch wire mesh to eliminate coprophagy (not done in our first experiments²). Daily records were made of running activity and food and water intake. From 4 to 14 rats were used for each carbohydrate. The first row of bar graphs in Figure 1 (without B₁) gives average survival times in days on each of the nine sugars and on no other food at all. Rats lived 46 days on levulose, 36 days on dextrose, only 11 days on lactose and 7 on galactose, and even fewer days on sorbose, sorbitol, and mannitol. They lived 6–7 days on no food at all.

In a slightly more complicated form this method was used to determine the effect of various vitamins on the utilization of the various foodstuffs.⁴,⁵ Thus, for instance, rats were offered a choice of a carbohydrate and a 0.025 per cent solution of thiamine hydrochloride and water, again in separate containers. When given access to B₁, rats on a single-food-choice of dextrose lived 74 days, just twice as long as on dextrose alone. (See last two rows of bar graphs in Fig. 1, with B₁ and in-
increase in per cent.) Vitamin B₁ had no effect on utilization of sorbose, lactose, sorbitol, and mannitol.

Figure 2.1 shows daily intake in grams (ten-day averages) of each of the nine sugars on the single food alone. The rats were kept on our stock diet for ten days before being started on dextrose. During the first ten days on the single foods, they ate large amounts of dextrose, maltose, levulose, and sucrose; then gradually less during successive ten-day periods. They ate very small amounts of sorbose, sorbitol, and mannitol. When given access to B₁ (see Fig. 2B), they ate definitely more levulose, sucrose, dextrose, and maltose, indicating that they could utilize more of these sugars. Their intake of the other sugars remained essentially unchanged.

Figure 3.1 shows body weight curves of the two groups of animals. On single-food-choice diets of dextrose or maltose, sucrose or levulose, body weight decreased
at a steady rate throughout the 30- to 40-day survival periods; Figure 3B shows that with access to B1 the rats lost weight at a much slower rate. On dextrose and B1 average weight decreased from 148 to 72 gm, or 48.6 per cent over the 70-day period.

Of most interest for the present purpose is the sugar, dextrose, on which, with access to B1, rats lived longest—74 days on the average and as long as 87 days in some instances.

Figure 4 shows daily food intake in grams, spontaneous running activity in number of revolutions of drum, vaginal smears, and weekly averages of body weight in grams for a rat on dextrose alone (A) and for a rat on dextrose and B1 (B). Graph B also shows daily intake of the 0.025 per cent B1 solution in milliliters. These are typical records for the two groups of rats. It is noteworthy that the rat on B1 remained very active throughout the first 60 days on the single-food diet and at death weighed only 70 gm as compared to the starting weight of 135 gm. During the 50- to 60-day period (ages 119-129), daily running activity averaged 14,500 revolutions. Up to within the last few days of life the rats showed no signs of deficiency—no loss or coarseness of hair, no defective teeth, no sores on the feet or skin. It is of special interest that in some animals four- to five-day cyclical changes in vaginal smears were present for several weeks or more. The animals were of course extremely emaciated. However, that they had not experienced any permanent damage was shown by the observation that when returned to the stock diet after 50-60 days on the single-food-dextrose-B1 diet, they quickly regained their original weight.
Fig. 4.—Graph showing daily records of spontaneous running activity as measured in revolving drum; food intake and body weight in grams; intake of a 0.025 per cent solution of thiamine hydrochloride; also a record of vaginal smears. (A) For a rat without access to B$_1$; (B) for a rat with access to B$_1$. 
Autopsy of rats that have been on the dextrose and B₁ choice diet for 50–60 days or more shows a complete loss of fat, marked atrophy and high transparency of all muscles, but only small, if any, atrophy of glands, and of course no detectable change in blood vessels. Adrenals, pancreas, gonads, thyroids, parathyroids, and hypophyses are all present, as are Peyer's patches on the intestines, and lymph, salivary, Harderian, hibernating, and preputial glands. The papillae and taste buds on the tongue appear normal. Microscopically the thymus is absent. The Hassall corpuscles may still be present but they are not visible to ordinary inspection. Spinal and peripheral nerves can be followed until they branch into the finest fibrils. Autonomic nerves can also be followed but not quite so well.

It is a truly remarkable fact of general biological interest that young growing rats can survive for such long periods—70 days or more—on the dextrose and B₁ diet that lacks all minerals, vitamins (except B₁), fat, and protein, when on a full diet lacking only one element, magnesium or calcium or phosphorus or vitamin A or B₆, etc., rats may develop deficiencies in only days or a few weeks.⁷

Without any food at all, rats die in six to seven days; that is, long before much fat and muscle tissue have been lost. The long survival times on this dextrose–B₁ diet allow ample time for all fat and much muscular tissue to disappear.

On the protein casein, rats survived for an average of 33 days; and when given access to B₁ for 55 days—66.7 per cent longer than on casein alone.⁸ These rats likewise lost all of their fat. We do not know whether they have any advantage over rats kept on the single-food dextrose and B₁.

Brief accounts will now be given of a few uses to which we have put this preparation during the past 15 years.

One of us (ORL) has used these preparations to study autonomic cells and fibers in the abdomen and pelvis. Injection of a dilute solution of methylene blue through the aorta immediately after death to perfuse the tissue makes it possible to follow autonomic fibers to their termination in the viscera.⁹ ¹⁰

Methylene blue has a special affinity for nerve cells, nerve fibers, and nerve endings. When injected into the blood stream it loses all its color owing to the absence of oxygen. Exposure to air brings out a deep blue stain in nerves, differentiating them from surrounding unstained fat-free tissues. The absence of fat seems to intensify staining with the dye as well as making nerves more accessible for dissection. Thus Figure 5A shows the large celiac ganglion and an extraordinary profusion of related nerves and plexuses. In Figure 5B the large pelvic ganglion in the male rat is seen in the lower part of the picture. Great numbers of strands of nerve fibers spread upward to innervate the prostate gland. With this technique detailed studies have been made of nerve supply to the bladder, prostate, seminal ducts, uterus, membranous urethra, penis, clitoris, and rectum.⁹ ¹⁰

It is most remarkable that in the highly transparent muscles of this preparation, nerve endings and muscle spindles can be clearly seen.

Another use has been made by one of us (EAP) for study of bone growth.⁶ ¹¹ It was pointed out that all changes produced on the single-food-choice diets are reversible, so far as we know, almost right up to the time of death. Returned to our stock diet, these animals eat large amounts, gain weight, and make a good recovery. It was found that after 50 days or more on the single-food-choice diet, bone growth of very young active growing rats shows a complete arrest: longitudinal
bone growth practically ceases; proliferative cartilage is reduced to a thin band trabeculae and cancellous tissue immediately under the cartilage are absorbed. These changes were observed chiefly in the upper end of the tibial bones. The section in Figure 6A of the upper tibia of a rat on a normal diet shows the wide band of proliferative cartilage ($C$), large amounts of cancellous trabeculae ($T$), and marrow ($M$). The section in Figure 6B of the tibia of a rat that had been on the dextrose and $B_1$ diet for 56 days shows the almost complete disappearance of all trabeculae, great narrowing of proliferative cartilage ($C$), and thin covering ($L$) of entire undersurface of cartilage. The section in Figure 6C of the bone of a rat that had been on the dextrose-$B_1$ diet for 58 days and on the recovery diet for 16 days gives some idea about how growth of bone can be followed in these preparations. Proliferative cartilage ($C$) has almost regained its normal width, a thick layer of trabeculae ($T$) has formed, and the transverse line ($L$) on the undersurface of the trabeculae is present but separated from the cartilage by a considerable distance. Thus, study of bones of rats killed at intervals after start of the recovery diet makes it possible to follow changes involved in decline of bone growth to a virtual zero and, when the full diet is restored, its complete rejuvenation, starting from zero to fullest development with all its complicated interlacing phenomena concentrated into a few days. Once the full diet was re-established, cellular activity appeared not only entirely normal, but showed a vigor to accelerate far in excess of that ever seen in animals kept under ordinary conditions.

Another use occurred in an entirely different area. It concerns the nature of so-called "Brown fat," or the hibernating gland, a leafy structure lying under each scapula and extending down around the rib cage. A heated controversy has been carried on for years as to whether this structure is fat or a gland.12 In normal animals it can scarcely be distinguished from regular fat. In this preparation, as was pointed out in an earlier paper,1 this structure retains its normal size long after disappearance of all fat, which demonstrates that it is not fat.
Fig. 6.—(A) Section of head of tibial bone of a normal control rat (M, marrow; T, trabeculae; C, cartilage). (B) Head of tibial bone of a rat that had been on a single-food-choice diet of dextrose and B₁ for 56 days (L, line along lower border of cartilage). (C) Head of tibia of rat that had been on the dextrose and B₁ diet for 58 days and on the recovery diet for 16 days.
This preparation offers an excellent opportunity for following blood vessels, even capillaries, in tissues ordinarily covered with fat.

We wish to draw special attention to the observation that lymph glands and Peyer’s patches and spleen retain their normal or nearly normal size, while the thymus disappears. Noteworthy is the freedom of these animals from any disease or infection. The fact that the thymus disappears does not of course suggest that it is fat tissue. It is well known that in starvation it disappears long before fat.

Other animals such as dogs, cats, and monkeys can undoubtedly be used. It was reported in the earlier communication that, as always happens in reviewing the literature, it was found that the great French physiologist, Magendie, had long before us made single-food-choice studies on dogs. He found that dogs lived as long as 30–36 days on exclusive diets of sugar, butter-fat, or gelatin, which is about the same length of time that rats survive on these foodstuffs without B1. With access to B1 dogs might live much longer, long enough to allow all or most all fat to disappear. Magendie of course did not know about vitamins and did not dissect his dogs.

Summary.—An ideal preparation was described for the dissection of spinal, peripheral, and autonomic nerves; of endocrine and other glands; and also for the study of bone growth. Rats were kept on a single-food-choice diet; that is, they had access to dextrose and a 0.025 per cent solution of thiamine hydrochloride (B1) and water, all in separate containers, and no other food or supporting substances. On this diet young rats survived 60–70 days and lost all fat. Their muscles showed marked atrophy and became transparent but their glands and nerves remained essentially normal. This preparation has been used to follow distribution of autonomic nerves to all the internal organs, to study bone growth, nerve endings, and muscle spindles, and to determine the nonfat nature of so-called “Brown fat,” or the hibernating gland.

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13 Magendie, F., Ann chem phys., 3, 66 (1816).