When the identical experiment was performed in excised, dead human skin, and in the intact skin of a living, anesthetized dog after the local injection of sodium azide, there was no fall in oxygen tension after the initial movement artifact. This evidence for the inability to utilize oxygen, noted in dead skin, and in living skin the cytochrome oxidase system of which had been blocked by sodium azide, bears out the usefulness of this method for the relative measurement of cutaneous oxygen consumption.

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Self-Selection of Diet in Relation to Audiogenic Seizures in Rats[1]

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It is known that some laboratory rats when subjected to sounds of high frequency exhibit convulsive behavior (*1, 2*). The pattern of such seizures is fairly uniform and consists of wild, undirected running, followed by tonic-clonic spasms and a comatose stage. Spontaneous seizures of a similar pattern have been reported in animals fed diets deficient in such substances as thiamine (*3*), pyridoxine (*4*), and magnesium (*5*). Likewise, supplementary feedings of thiamine hydrochloride have been found to render rats selectively bred for seizure susceptibility increasingly resistant to the sound-induced convulsions (*6*).

The similarity between patterns of convulsive seizures resulting from inadequate diets and those that occur under auditory stimulation has made it difficult to determine the etiology of the latter type of seizure. It occurred to us that utilization of a self-selection technique similar to that employed by Richter (*7*) might enable us to detect subjects in our colony whose susceptibility to sound-induced convulsions was conditioned by dietary factors. Such a technique might prove of special value in those cases lacking observable evidence of nutritional deficiency.

Sixty-five albino rats, males averaging 45 days of age at the start of the experiment, were studied. Wherever possible littermates were used, and the split-litter technique employed. Essentially the same method of auditory stimulation was employed as in previous experiments on the production of convulsions in rats (*8*).

In order to determine the incidence of audiogenic fits, the subjects were exposed to 3 min of stimulation with a heavy-duty electric bell daily for 14 days prior to the experiment proper. During this period all animals were maintained on laboratory-prepared stock diet, consisting per 1,000 g of diet of the following ingredients:

Graham flour	725 grams
Skim milk	100 "
Casein	100 "
Calcium carbonate	15 "
Sodium chloride	10 "
Butterfat	50 "

Twenty rats, selected on the basis of their degree of susceptibility to sound-induced seizures, composed the experimental group. Ten of the rats showed fits in excess of 50% of the times tested and were designated consistently susceptible animals. The remaining 10 experimental subjects had seizures less than 25% of the times tested and were considered sporadically susceptible rats. Forty-five rats constituted the control group. Of these, 15 showed the seizures consistently, 15 had sporadic fits, and 15 failed to have any seizures.

Three diets were used in the experiment proper: the stock diet referred to above, commercial Purina Dog Chow Checkers, and a self-selected diet. The latter consisted of the following:

Solutions presented in 100-ml graduated inverted bottles affixed to especially constructed living cages:

1% solution of potassium chloride
2% solution of calcium lactate
3% solution of sodium chloride
4% solution of sodium hydrogen phosphate
0.02% solution of vitamin B-1
0.02% solution of vitamin B-6
0.01% solution of calcium panothenate
0.1% solution of nicotinamide
0.5% solution of choline chloride
0.00125% solution of riboflavin
Distilled water
Olive oil
Cod liver oil

In solid form, presented in nonspillable food cups:

Dextrose
Vitamin-free casein

All the experimental animals were placed for 14 days on each of the diets in random order. Tests for susceptibility to audiogenic seizures were given for 3-min periods daily. Of the control rats, 5 animals from each of the resistant, consistently susceptible, and sporadically susceptible groups were maintained throughout the 42-day experimental period on each of the 3 diets. All controls were exposed to 3 min of auditory stimulation daily.

Daily nutritional intakes, self-selection choices, and responses to sound stimulation were recorded. Weekly fluctuations in body weight were measured.

Among the experimental animals consistently susceptible to sound-induced seizures, 2 animals failed to show the fits when placed for 14-day periods on the self-selected diet. Both these subjects were found to have atypical selections of thiamine. One of the rats averaged 2.5 mg/day, the other 4.0 mg/day, intake of thiamine hydrochloride. These amounts were in excess of the normal

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requirements for animals of the age and body weight of these 2 subjects (*9*). On both the stock diet and Purina Chow these experimentals showed audiogenic seizures consistently. Of the sporadically susceptible experimentals, one animal failed to have sound-induced seizures while on the self-selected diet. This rat was found to have an average daily intake of magnesium chloride that amounted to 3 mg. Compared with the magnesium requirements of rats of the age and body weight of this subject, this amount can be considered excessive. The animals continued to show fits sporadically when placed on either stock or Purina diet. Three of the consistently susceptible controls and one of the sporadically susceptible control rats failed to have audiogenic fits during the 42 days on the self-selected diet.

In every instance the controls whose seizures were alleviated by the self-selected diet showed excessive intakes of thiamine hydrochloride. The approximate average amount of this vitamin consumed per rat per day amounted to 5.0 mg. In general, in the cases where self-selection alleviated seizure susceptibility, the rats failed to show the seizures after an average of 8 days on the diet.

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Phenyl Phenaceturate from the Decomposition of Penicillin in the Presence of Phenol

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Aqueous solutions of potassium penicillin G containing sodium citrate as buffer and phenol as a preservative may be kept under refrigeration for relatively long periods of time without undergoing serious deterioration. However, at room (25° C) or higher temperatures such solutions have been observed to deposit a colorless crystalline precipitate. This precipitate has been identified as phenyl phenaceturate.

A solution containing 31.6 g of crystalline potassium penicillin G, 2.5 g of phenol, and 2.5 g of sodium citrate in 1 liter of water was kept at 37° C. After 24 hr a slight crystalline precipitate was present and after 48 hr about 2 g of the precipitate had separated. No more appeared to be formed on longer standing at 37° C. The long needlelike crystals were collected and air-dried. They were insoluble in water, sparingly soluble in ether, readily soluble in acetone, and easily recrystallized from alcohol, mp 132°–133° C. Analysis showed the presence of 71.39% carbon, 5.85% hydrogen, and 5.49% nitrogen. Calculated values for phenyl phenaceturate ($C_{15}H_{15}NO_3$) are 71.36% carbon, 5.62% hydrogen, and 5.20% nitrogen. A sample of the compound was saponified by warming with dilute alcoholic sodium hydroxide solution. After evaporation of the alcohol and acidification of the residue with hydrochloric acid, there was present a strong odor of phenol, and phenaceturic acid crystallized from the solution. It was identified by mp 142°–143° C and mixed melting point with an authentic sample. The phenaceturic acid was also characterized by the identity of its x-ray powder diagram with that of an authentic sample.

The phenyl phenaceturate obtained from penicillin was further identified by finding that its x-ray powder diagram and infrared absorption spectrum were identical with those of a synthetic sample of phenyl phenaceturate. The synthetic sample was prepared as follows: Phosphorus tribromide, 7.1 g (0.026 mole), was added to a solution of 5.5 g (0.028 mole) of phenaceturic acid in 50 ml of dry dioxane. The resulting crystalline precipitate of 2-benzyl-4(5)-oxazalone hydrobromide (*1*) was centrifuged and washed with anhydrous ether. To the solid was added 5.3 g (0.056 mole) of phenol, and the mixture was heated at 80°–90° for 1 hr, after which it was poured into ice water. The oil that separated eventually crystallized. This was dissolved in ethyl acetate; the solution was washed with aqueous sodium bicarbonate solution, dried, and evaporated in vacuum until a crystalline precipitate separated. The product, phenyl phenaceturate, was recrystallized from alcohol—yield, 1.25 g (20%); mp 132°–133°—and mixed with the product obtained from penicillin as described above, mp 132°–133°.

It is not surprising to find derivatives of phenaceturic acid resulting from the decomposition of penicillin G (*2*), nor is it particularly surprising that the azlactone ring of penicillin is apparently opened by phenol. The azlactone ring is similarly opened by methanol, ethanol, and other alcohols, which form the corresponding esters of penicilloic acid (*3*). However, it was surprising to find that phenyl phenaceturate was formed from penicillin G and phenol in aqueous solution only when a buffer was present. In the absence of a buffer the solutions turned yellow on long standing or warming, but deposited no precipitate. Potassium phosphate, as well as sodium citrate, as described above, was effective in promoting the formation of phenyl phenaceturate in solutions containing phenol and penicillin G.

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