Reports

Occurrence of l-Quinic Acid in Tobacco Leaves

Quinic acid has been isolated and identified as a constituent of various fruits and grasses (*1–3*). This report describes the isolation of *l*-quinic acid from mature green leaves of *Nicotiana tabacum* var. Connecticut shade-grown (*4*). Quinic acid is a normal constituent of the leaf, and preliminary data suggest that it may serve as a precursor of chlorogenic acid.

Separation of quinic acid from the major organic acid, malic acid, was achieved by displacement chromatography (*2, 3*). A water extract (*5*) of 30 g of dried green tobacco leaves was decolorized with 8 g of Darco G-60 (*6*) and passed successively through a column of Dowex 50-X8 (100 to 200 mesh, hydrogen form) (*7*) 15 cm by 3.5 cm^2 and a column of Dowex 1-X4 (200 to 400 mesh, acetate form) (*7*) 17 cm by 3.5 cm^2. The columns were washed with water, and the organic acids were displaced from the Dowex 1 with 0.1*N* HCl. Fractions of 10-ml volume were collected and examined by paper chromatography. Malic acid first appeared in fraction 20; fractions 4 to 17 contained a mixture of acids, including quinic and D-glyceric acids (*8*). These fractions were pooled, and the quinic acid was separated from the other acids by elution from the column of Dowex 1 with 1*N* acetic acid, as has been previously described (*8*). The quinic acid occurred in fractions 32 to 44, immediately preceding glyceric acid. It was isolated from these fractions essentially as described by Anet and Reynolds (*3*), except that the residue after the initial evaporation was dissolved in water and dried in a vacuum to remove the last traces of acetic acid, and the final crystallization from water was omitted.

The acid isolated from tobacco leaves exhibited the same chromatographic behavior on ion-exchange columns and on filter paper as authentic *l*-quinic acid. It is eluted just ahead of glyceric and glycolic acids from Dowex 1 columns in the acetate form (*5*) and has an R_F of 0.30 in an ether-formic acid-water solvent system (*5*) and an R_F of 0.48 in propanol-ammonia-water (60/30/10) (*9*).

The melting point (165° to 166°C), specific rotation ($[\alpha]_D^{20} = -42.5°$; concentration, 0.8 g/100 ml), and infrared spectra were identical with those of authentic *l*-quinic acid. A mixture showed no depression of the melting point. The isolated sample turned brown just before melting, presumably because of a slight contamination with impurities from the resin.

This specimen of quinic acid was isolated from tobacco leaf samples which had been dried at 80°C for 3 hours. Chlorogenic acid (a depside between quinic and caffeic acids) is present in tobacco leaves, and drying of the leaves results in extensive losses of this acid (*10, 11*). Thus, it was possible that all or part of the quinic acid present was a product of the decomposition of chlorogenic acid. As a test of this point, three identical samples of green leaves were extracted by different techniques, and the chlorogenic and quinic acid contents were compared. One sample was air-dried at 80°C and extracted with hot water, the second was plunged immediately after picking into boiling water, and the third was plunged into boiling 80 percent ethanol. The latter two techniques have been shown to prevent the loss of chlorogenic acid (*11*). The leaf material was held at the boiling point for several minutes, was cooled, and was homogenized in order to extract the acids.

The quinic acid in the extracts was determined by ion-exchange chromatography on Dowex 1 columns in the acetate form (*5*). The fractions containing the quinic acid were taken to dryness at 50°C while air was blown on the surface (*5*), water was added to the residue, and the mixture was again dried before titration with dilute alkali. Yields in excess of 100 percent are obtained if this second drying step is omitted. Chlorogenic acid was estimated by measurement of the optical density of the appropriately diluted solutions at 324 mμ (*12*).

All three samples contained approximately the same quantity of quinic acid, despite the fact that an 80-percent loss of chlorogenic acid occurred in the air-dried sample. In fact, the quinic acid content of oven-dried samples is usually less than that of comparable samples extracted directly by boiling solvents. Paper chromatography of the extracts (*11*) revealed a single, rather ill-defined spot whose R_F was identical with that of authentic chlorogenic acid. The relative intensities of the spots provided qualitative confirmation of the spectroscopic results.

These data provide proof of the occurrence of *l*-quinic acid in the green tobacco leaf. For reasons that are not known, the quantity present varies widely from sample to sample, ranging from a trace to as much as 3.5 percent of the dry weight. The chlorogenic acid content of tobacco also fluctuates over a wide range (*11*).

The metabolic role of quinic acid is not known. Oxidation yields citric and malonic acids, and one possible pathway for utilization could be by way of citric acid (1). Quinic acid could also serve as an intermediate in aromatic biosynthesis (*13*), or it could react with caffeic acid to form chlorogenic acid.

A few preliminary experiments have been carried out in an attempt to define this role. When leaf disks were floated on 0.2*M* quinic acid solutions at *p*H 3.5 or 5 for 24 hours in the dark, they took up the acid readily, and about 25 percent of the uptake was utilized during this period. There was no evidence of a direct conversion to citric acid, but there was an apparent increase in chlorogenic acid of 20 percent at *p*H 5 and of 37 percent at *p*H 3.5. However, these figures were obtained from the increase in optical density at 324 mμ, and recent work (*11*) has reemphasized the necessity for caution in interpreting such spectroscopic data. Further studies await the development of more reliable techniques for the assay of chlorogenic acid and related compounds.

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4. I am indebted to H. B. Vickery for many helpful discussions and suggestions throughout the course of this work and also to the National Science Foundation for providing part of the financial support.

All technical papers and comments on them are published in this section. Manuscripts should be typed double-spaced and be submitted in duplicate. In length, they should be limited to the equivalent of 1200 words; this includes the space occupied by illustrative or tabular material, references and notes, and the author(s)' names(s) and affiliation(s). Illustrative material should be limited to one table *or* one figure. All explanatory notes, including acknowledgments and authorization for publication, and literature references are to be numbered consecutively, keyed into the text proper, and placed at the end of the article under the heading "References and Notes." For fuller details see "Suggestions to Contributors" in Science 125, 16 (4 Jan. 1957).

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Physiological Response to Air Exposure in Codfish

When diving mammals and birds submerge they exhibit a series of characteristic phenomena. The heart rate slows down. Lactic acid accumulates in the muscles, but only a very little leaks out into the circulation, because blood flow through the muscles is strongly depressed. This part of the picture is virtually the same whether the animal is quiet or active during the dive. In the recovery after the dive, circulation increases, and the lactic acid from the muscles pours into the blood. These and other adjustments are strikingly developed in many diving mammals and birds and have been found, in a more or less pronounced degree, in every warm-blooded animal where such investigations have been made. Man also frequently develops a pronounced bradycardia during diving, even while swimming vigorously (*1*).

Unpublished experiments performed at Woods Hole, Mass., several years ago indicated that similar mechanisms operate in dogfish (*Squalus*) when they are taken out of water, and these observations led to the present investigation (*2*) on codfish (*Gadus callarias*) at the Biological Station at Drøbak, Norway.

Heart rate and lactic acid in blood and muscles were determined (i) when the fish were resting quietly in the water, (ii) when they were taken out of water and left struggling in air for 4 minutes, and (iii) when they were placed back in water for recovery. Heart rates were taken on the same fish throughout the sequence, but lactic acid samples were, in all cases, taken from different fishes and give therefore a statistical picture of the sequence. Heart rates were obtained by means of an electrocardiograph. Blood or muscle samples for lactic acid determinations were taken immediately after removing the fish from the water and were analyzed colorimetrically (*3*).

When the fish was taken out of water the heart rate dropped within a few seconds to half or less of normal (Fig. 1), and this bradycardia persisted even through violent muscular activity. When the fish was put back in water the normal pulse rate was rapidly restored.

In the muscles the lactic acid rose to a maximum during the air exposure and fell as soon as the fish was put back into the water. In contrast, the blood lactic acid remained low during the air exposure but rose in the recovery period and reached a maximum after some 5 to 15 minutes. As recovery proceeded, the lactic acid in blood and muscles slowly dropped back to normal (Fig. 1).

These events, produced by taking the fish out of water, are strikingly similar to those found when mammals and birds are submerged, and they very probably indicate the same sort of protective mechanisms against asphyxia—namely, a circulatory bypass of the muscles, which are thereby left isolated to operate on their own anaerobic resources. The bradycardia is very probably a direct consequence of the restricted peripheral blood flow, for at least in some diving mammals, the main arterial blood pressure does not drop during the bradycardia, which suggests that normal blood flow may be maintained in organs less capable of anaerobic functions than the muscles. The similarity of this mechanism to those present in diving mammals goes even further, inasmuch as bradycardia in the fish can be induced, just as in a seal, simply by frightening the animal. This "diving reflex" might seem to be an excellent idea for a flying fish, but how it could ever benefit a codfish is an evolutionary puzzle.

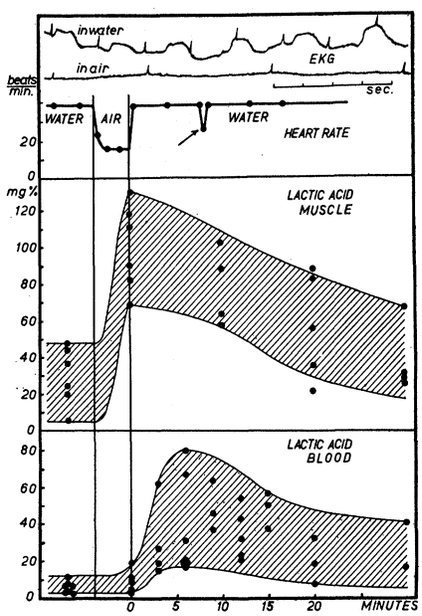

Fig. 1. Electrocardiograph tracings, heart rate, and content of lactic acid in muscles and blood of the codfish. The fish was taken out of water for 4 minutes. At arrow, hand was placed around submerged animal.

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Irregular Maintenance Schedules and Drive

In animal experimentation the hunger drive is usually defined in terms of hours of food deprivation. Attention is rarely paid to the animal's prior history of deprivation. The deprivation history of an appetitive drive is controlled by means of a maintenance schedule—that is, the schedule of eating and deprivation intervals to which the animal is subjected over a period of time. If maintenance schedules affect subsequent behavior, then any variation from laboratory to laboratory may account for discrepant experimental findings.

Although it is known that irregular reinforcement profoundly affects animal behavior and is also a prominent feature of learning conditions outside the laboratory, almost nothing is known about the effects of irregular maintenance schedules. The present set of experiments represents a first attempt to study the effects of irregular food maintenance schedules on learning in the albino rat (*1*). It is expected that irregular schedules would elevate the drive effect of the deprivation interval used at time of testing.

In experiment 1, two groups of 70-day-old rats were placed on the following food maintenance schedules: a regular (R) group ($N = 5$) received 12 g of mash every 12 hours, and an irregular (I) group ($N = 6$) averaged the same amount of daily food intake but experi-