days (*12*). Under these conditions ripening again was effected in 4 days when the partial pressure of ethylene was increased to 6×10^{-6}, so it can be concluded that more ethylene is required to ripen fruits when the concentration of oxygen is lowered. This effect and a reduction in ethylene content are offered as an explanation for increased storage life at subatmospheric air pressures.

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7. For example, there is an exact correlation between the partial pressure of ethylene within an apple and the absolute pressure of the atmosphere in which the apple is situated, even though the rate of ethylene formation does not vary with atmospheric pressure.
8. Ripening is delayed by a factor of more than 4 when fruits are stored in air at 250 mm-Hg instead of 760 mm-Hg (Table 1), whereas in oxygen at 180 mm-Hg the delay is less than a factor of 2 relative to the control in air at atmospheric pressure.
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11. In these experiments reducing the pressure had its characteristic effect, retarding ripening in the absence of ethylene from 18 days in air at 760 mm-Hg to 31 days in oxygen at 180 mm-Hg.
12. In air at atmospheric pressure without ethylene present the fruits ripened in 10 days in this experiment. At a pressure of 250 mm-Hg ripening would occur in approximately 40 days (see Table 1), but under these conditions ripening required only 16 days when ethylene was included. It is clear that the gas enhanced ripening considerably even though it failed to cause its maximum effect.
13. Patent applied for.
14. This research was supported by a grant from the United Fruit Co.

26 April 1966

Inhibition of Protein Synthesis: A Mechanism for the Production of Impaired Iron Absorption

Abstract. *Treatment of rats with cycloheximide results in a defect in intestinal iron transport. A similar defect occurs after the parenteral administration of iron. Under both conditions there is impaired uptake of iron into the mucosal cells as well as defective transfer from the intestinal mucosa. It is suggested that the interference in iron transport may be due to a deficiency of an unidentified carrier substance.*

The intestinal absorption of iron (*1–4*) involves at least two steps: (i) mucosal uptake of iron from the intestinal lumen and (ii) transfer of iron from mucosal cells to the plasma. These studies indicate that the intestinal mucosa is important in controlling iron absorption. The actual regulatory mechanisms remain poorly understood, however. Possibly an unidentified carrier substance is important in the transfer of iron from the intestinal mucosa.

Our study shows that inhibition of protein synthesis in the rat by the antibiotic cycloheximide (*5*) is associated with impairment in the absorption and transport of iron from the intestine.

Female Wistar rats (140 to 160 g) were fasted for 16 hours prior to study. The animals were separated into four groups: (i) controls; (ii) rats given a low-iron diet (Nutritional Biochemicals) for 2 weeks prior to study; (iii) rats given an intramuscular injection of Imferon (25 mg) 2 weeks prior to study; and (iv) rats given an intraperitoneal injection of cycloheximide (5 mg per kilogram of body weight) 3 hours prior to study. After the animals were lightly anesthetized with ether, the proximal 16 to 20 cm of small bowel was isolated between silk ligatures, care being taken not to obstruct blood flow. The rats received an intraduodenal injection of $Fe^{59}SO_4$ (50 μg containing 2×10^6 disintegrations per minute). After 30 minutes the isolated loops were excised, the contents were washed out with 50 ml of 0.9 percent NaCl, and the mucosa was removed by scraping. Portions of the loop contents and the mucosa were assayed for radioactivity in a well-type scintillation counter.

Data on the relative rates of absorption of iron in the four groups of animals were determined by measuring the mucosal uptake and mucosal transfer of $Fe^{59}SO_4$ (Table 1). The mucosal uptake of radioiron (A) was determined by subtracting the amount of radioactivity recovered in the loop contents from the amount injected. The amount of radioiron remaining in the mucosa (B) was measured directly. The mucosal transfer of radioiron (A − B) represents the difference between mucosal uptake and the amount remaining in the mucosa. Mucosal transfer represents net absorption. In control rats there was a mucosal uptake of 65 percent and transfer of 37 percent of the injected dose. In rats previously on a low-iron diet there was a significantly increased mucosal uptake (83 percent) and transfer (56 percent) of radioiron. In contrast, in rats previously injected with Imferon there was a decreased mucosal uptake (52 percent) and a markedly reduced transfer (16 percent) of radioiron. Administration of cycloheximide decreased mucosal uptake (29 percent) and transfer (12 percent) of radioiron.

The amount of radioiron remaining in the mucosa divided by the mucosal uptake, B/A, also reflects the interference in iron transport induced by treatment with iron or cycloheximide. In control animals 42 percent of the iron taken up by the mucosa was still present in the mucosa after 30 minutes, while only 33 percent remained in iron-deficient animals. In contrast, in animals treated with iron or cycloheximide there was a significant accumulation of iron in the mucosa, with 69 and 59 percent, respectively, of the iron taken up by the mucosa still there after 30 minutes.

To confirm that cycloheximide blocks iron absorption, the absorption

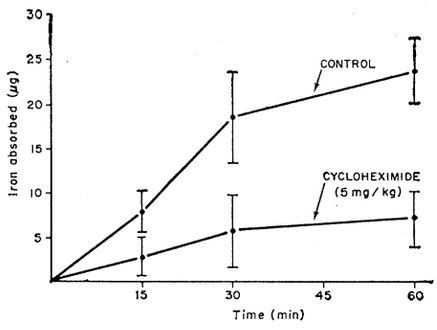

Fig. 1. Absorption of $Fe^{59}SO_4$ (50 μg containing 2×10^6 disintegrations per minute) from isolated intestinal loops in control and cycloheximide-treated rats. The values shown represent the mean ± 1 S.D.

Table 1. Mucosal uptake and mucosal transfer of $Fe^{59}SO_4$ (50 μg containing 2.0 $\times$ 10^6 disintegrations per minute) from isolated intestinal loops. A, Mucosal uptake; B, remaining in mucosa; A—B, mucosal transfer.

Group	Rats (No.)	A	B (% ± S.D.)	A − B	B/A (%)
Control	20	64.8 ± 11.4	27.8 ± 9.3	37.0 ± 10.9	42
Iron-deficient	10	82.9 ± 5.8	27.3 ± 5.1	55.6 ± 7.1	33
Iron-loaded	14	51.6 ± 11.5	35.9 ± 12.6	15.7 ± 8.2	69
Cycloheximide	18	28.8 ± 14.1	17.0 ± 6.8	11.8 ± 8.2	59

of iron was compared in control and cycloheximide-treated rats. Isolated intestinal loops were removed 15, 30, or 60 minutes after injection of radioiron, and the mucosal uptake and mucosal transfer were determined as described above. The amount of iron absorbed was calculated on the basis of the mucosal transfer and the dose of radioiron, 50 μg (Fig. 1). In control animals iron absorption progressively increased; 23.5 μg was absorbed in 60 minutes. In contrast, in cycloheximide-treated rats there was a minimal increase in iron absorption over a 60-minute period with only 7.4 μg being absorbed.

The absorption of glucose from isolated intestinal loops was compared in control and cycloheximide-treated rats. After an intraduodenal injection of glucose (1.5 g per kilogram of body weight) the increase in blood glucose was similar in both groups. The maximum values for blood glucose were 216 ± 50 (1 S.D.) and 210 ± 42 mg per 100 ml, in control and cycloheximide-treated animals respectively. The normal absorption of glucose and the minimum changes in the intestinal mucosa detected by light and electron microscopy suggest that the impaired iron absorption associated with the administration of cycloheximide is not a nonspecific effect due to tissue damage. In this connection, it is pertinent that rats treated wtih acetoxycycloheximide absorbed triglycerides of fatty acids of medium-chain length at nearly the same rate as controls (*6*).

Several studies have indicated that iron is present in the intestinal mucosa in two forms: (i) nonprotein-bound iron which is transported rapidly across the mucosa and (ii) iron which is slowly transported across the mucosa (*2, 3, 4*). Two groups of investigators (*4, 7*) have suggested that the slowly transported iron has been incorporated into mucosal ferritin. The nature of the rapidly transported iron has not been clearly defined.

The mechanisms regulating iron absorption remain poorly understood. It has been proposed that the intestinal mucosa receives a "signal" and proportionate amounts of iron are either rapidly transported across the mucosa or incorporated into ferritin with a resultant delay in transport (*4*).

Our data are in accord with those which show that iron absorption is increased by a diet deficient in iron (*4, 8*) and that the mucosal uptake and transfer of iron is significantly impaired by iron loading (*2, 4*). A similar defect in the mucosal uptake and transfer of iron was produced by treatment with cycloheximide. The mechanism of the impaired iron absorption resulting from these treatments is unknown. Our data suggest that a specific carrier substance transfers iron in the mucosa. Such a substance could be a protein, a polypeptide, or any specific amino acid which forms complexes with iron (*3*). Iron loading might saturate this carrier or repress its synthesis. It is possible that an agent such as cycloheximide impairs synthesis of the carrier. Furthermore, a carrier substance might be important in the entry of iron into the mucosal cell as well as the transport of iron from the cell. This hypothesis could account not only for the decreased mucosal transfer, but also for the decreased mucosal uptake of iron noted after treatment with iron or cycloheximide.

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Simultaneous Changes in Visual Separation Threshold and Voltage of Cortical Alpha Rhythm

Abstract. *An automated psychophysical method allows a human subject to track his own separation threshold for two flashes delivered in sequence without interfering with the simultaneous recording of the alpha rhythm of his electroencephalogram. A systematic rise in this threshold is observed when the alpha voltage declines from its normal alert level. This suggests that an increase in the minimum resolvable temporal sequence may contribute to the development of the negative time errors associated with drowsiness and sleep.*

A previous study (*1*) demonstrated that as the alpha voltage, shown in an electroencephalogram (EEG), declines from a general alert level there is an increasing tendency to underestimate the passage of time, that is, to make negative time errors. A mechanical timepiece making negative time errors would ordinarily be described as "losing time," a fault indicating that there is something wrong with either the mechanism generating its basic temporal units or the mechanism summing these units, or both. The present experiment was designed to explore the first of these two possibilities in the case of human perception of change during drowsiness, namely, that there may be some distortion in the perception of the smallest units of change. The question asked is not how effectively is time spanned but rather how effectively is time resolved? Specifically, are the fluctuations in the *minimum resolvable sequence* of photic stimuli systematically related to variations in the alpha voltage shown in the EEG?

If the second of two flashes in sequence follows too soon after the first, the subject reports only one flash in-