wave is characteristic for the fission of hydrogen bonds and unfolding of the protein molecule. Unfolding is reversible and the first wave is therefore unchanged if the urea concentration is reduced after denaturation.

During irradiation in air-free media no unfolding occurs but aggregation gives rise to molecules of high M as reported by RIDEAL and ROBERTS[5] who also found that photooxidative decomposition occurs when albumin is irradiated in the presence of air. Since it is known that larger molecules give smaller catalytic waves, it can be concluded that the smaller waves (h_1) in irradiated air-free BSA solutions are caused by aggregation while the higher waves in the presence of air are the result of photo-oxidation.

The second wave in irradiated solutions is probably the result of cross-linking reactions between newly formed sulfhydryl and remaining disulfide, although the correctness of this assumption cannot be demonstrated by irradiation experiments with BSA solutions in the presence of mercuric chloride which, if present in sufficient amounts, gives rise to precipitation of protein during irradiation.

The polarographic results obtained with irradiated and urea denatured BSA lead to the conclusion that under proper experimental conditions the height of the first peak is characteristic for the secondary structure and molecular size of the protein, while the appearance of a second wave is indicative for cross-linking reactions.

Guanidine hydrochloride has a specific effect on the catalytic protein waves and can therefore not be used for a polarographic study of denaturation effects[6].

Zusammenfassung. Serumalbumin von Rindern wurde in 2 verschiedenen Konzentrationen mit UV-Licht bestrahlt und die polarographisch-katalytischen Wellen mit denen von Albumin verglichen, das zuvor mit Harnstoff behandelt worden war.

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Department of Chemistry, Marquette University, Milwaukee (Wisconsin 53233, USA),
August 22, 1966.

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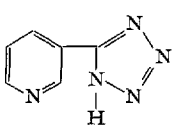
[6] This investigation was supported by the U.S. Army, Medical Research and Development Command, Department of the Army, under Research Contract No. DA-49-193-MD-2146.

5-(3-Pyridyl)tetrazole, a Potent and Long-Acting Lipolysis Inhibitor

The administration of nicotinic acid to man lowers the level of plasma free fatty acids (FFA)[1,2]. The depression of plasma FFA is of short duration and is followed by a rise of plasma FFA above the normal fasting level. Also, nicotinic acid in man, as well as in animals, blocks the catecholamine stimulated mobilization of FFA in vivo, as well as the release of FFA from adipose tissue in vitro. CARLSON and ORÖ[1,3] have suggested that nicotinic acid lowers plasma cholesterol and triglycerides by inhibiting FFA release from adipose tissue.

The rapid metabolic inactivation of nicotinic acid could account for the short duration of plasma FFA lowering. GINOULHIAC et al.[4] have shown that nicotinic acid reaches a peak blood concentration 1 h after the oral administration of 500 mg. This dose of nicotinic acid is cleared from the blood of humans within 4 h. Only 18% of the dose could be recovered unchanged in the urine after 24 h. These observations suggest that an important factor in the removal of nicotinic acid from blood is its conversion to inactive metabolites. The brief sojourn of nicotinic acid in the blood appears adequate to explain the short duration of nicotinic acid-induced FFA depression in man. A lipolysis inhibitor of the same magnitude of activity in vitro as nicotinic acid, but of greater metabolic stability, would be expected to depress plasma FFA for a longer period of time.

5-(3-Pyridyl)tetrazole (I)[5], has some of the important salient structural features of nicotinic acid, i.e. a pyridine nucleus with an acidic function at the 3 position. HERBST and WILSON[6] have shown that 5-substituted tetrazoles are acidic. More importantly, the tetrazole function is metabolically stable. For example, about 75% of a dose of pentylenetetrazole (Metrazol) can be isolated from rat urine unchanged[7]. I was prepared by an improved procedure for the synthesis of 5-substituted tetrazoles[8]. As expected, the apparent ionization constant of I ($pK_a = 4.1$) was quite similar to that of nicotinic acid ($pK_a = 4.5$)[9]. 5-(3-Pyridyl)tetrazole showed lipolysis inhibitory activity which was similar in some respects to nicotinic acid, but differed significantly and interestingly in other aspects.



I

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[4] E. GINOULHIAC, L. T. TENCONI and F. M. CHIANCONE, Nature *193*, 948 (1962).
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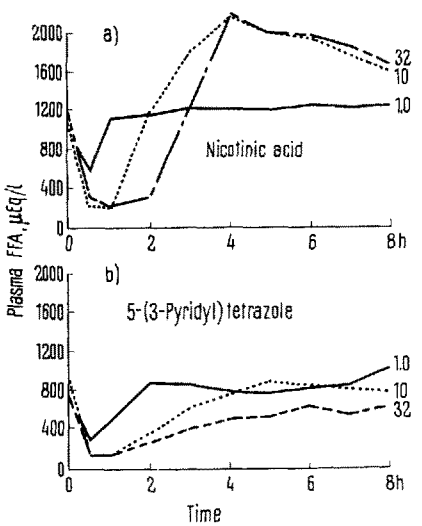
The inhibition of norepinephrine-induced release of fatty acids was studied with epididymal adipose tissue taken from male Sprague-Dawley rats, 180–240 g, fed ad libitum. The tissue was placed in freshly aerated Krebs-Ringer bicarbonate buffer, pH 7.4, and minced with scissors into pieces weighing approximately 10 mg. Each experimental flask contained 3 ml of freshly aerated (95% O_2-5% CO_2) Krebs-Ringer bicarbonate buffer and 200 ± 3 mg (mean ± standard deviation) of adipose tissue. Bovine plasma albumin, fraction IV, 1%, was used as a fatty acid acceptor in the incubation medium. Adequate norepinephrine (20–30 ng/ml) was added to the incubation mixture to elicit a 50% maximum fatty acid release. The compounds under test were added at appropriate concentrations. The experimental flasks were stoppered, aerated with 95% O_2-5% CO_2 for 10 min and incubated at 37 °C for 3 h on a Dubnoff metabolic shaker. After incubation, aliquots were removed for fatty acid analysis by the method of DOLE[10]. The effects of the inhibitors were expressed in terms of % inhibition of the FFA release produced by norepinephrine.

The Table compares the inhibitory effects of nicotinic acid and 5-(3-pyridyl)tetrazole (I) on the norepinephrine-stimulated release of FFA from adipose tissue in vitro. These data show that nicotinic acid is about 1000 times more potent than I in this system. The concentration of nicotinic acid required to produce 50% inhibition is between 10^{-7} and $10^{-6}M$, while the concentration of I required for a similar effect is between 10^{-4} and $10^{-3}M$. We conclude, therefore, that the replacement of the carboxyl function in nicotinic acid by the tetrazole moiety causes a substantial decrease in intrinsic lipolysis inhibitory activity.



Time course of the effects of (a) nicotinic acid and (b) 5-(3-pyridyl)-tetrazole (I), 1.0, 10 and 32 mg/kg, i.v., on the fasting plasma FFA values of adult mongrel dogs.

Inhibition of norepinephrine-induced FFA release from adipose tissue in vitro

Concentration	% Inhibition[a]	
	5-(3-Pyridyl)-tetrazole (I)	Nicotinic Acid
$10^{-3}M$	59 ± 3.0	69 ± 4.4
$10^{-4}M$	31 ± 1.4	68 ± 3.3
$10^{-5}M$	27 ± 1.6	68 ± 4.9
$10^{-6}M$	19 ± 3.6	66 ± 5.0
$10^{-7}M$	8 ± 2.4	28 ± 4.1

[a] Mean ± S.D. of 5 experimental flasks.

Nicotinic acid depressed plasma FFA after intravenous administration to normal fasted dogs (Figure, a). A submaximal depression of plasma FFA was observed at a dose of 1 mg/kg. This response was intensified and prolonged by an increase in dose to 10 mg/kg. Increasing the dose beyond 10 mg/kg prolonged, but did not intensify, the FFA decrease. A rise of plasma FFA above the basal level, plasma FFA rebound, was observed after doses of 10 and 32 mg/kg. This rebound phenomenon is currently under investigation. The duration of the FFA depression was approximately 1, 2 and 3 h after i.v. administration of 1, 10 and 32 mg/kg, respectively. The elevation of plasma FFA, i.e. rebound, after doses of 10 and 32 mg/kg, extended over the remainder of the 8 h observation period. If one takes into account the plasma FFA rebound phenomenon, it is obvious that single doses of 10 or 32 mg/kg of nicotinic acid in the dog produce supernormal levels of circulating FFA.

When a dose of 1 mg/kg of 5-(3-pyridyl)tetrazole (I) was administered to dogs a near maximal decrease in plasma FFA was seen (Figure, b). An increase in dose to 10 mg/kg intensified and prolonged the decrease; while an increase in dose to 32 mg/kg did not intensify, but did prolong the FFA depression. The duration of the FFA depression was approximately 2, 5 and 8 h after i.v. administration of 1, 10 and 32 mg/kg, respectively. No indication of FFA rebound was observed and it is obvious that the overall response to I involves a net decrease in fatty acid mobilization.

The data presented indicate that I bears a qualitative resemblance to nicotinic acid in several characteristics, including lipolysis inhibition in vitro and plasma FFA depression in vivo. Quantitative differences exist in the very much reduced in vitro potency of I and the considerably longer duration of FFA depressing activity, without rebound, of I in vivo. The improved duration of action of 5-(3-pyridyl)tetrazole over nicotinic acid, in spite of a decreased intrinsic activity in vitro, is probably attributable to the greater metabolic stability of I[11]. Additional actions of I are currently under investigation.

Zusammenfassung. Es wird gezeigt, dass 5-(3-Pyridyl)-Tetrazol und Nikotinsäure die Menge der unveresterten Fettsäuren (UFS) bei fastenden Hunden erniedrigen. Die Tetrazolanalogverbindung der Nikotinsäure hat eine längere Wirkungsdauer als Nikotinsäure, obwohl ihre antilipolytische Wirksamkeit in vitro viel geringer als die der Nikotinsäure ist. Die verlängerte Wirkung von 5-(3-Pyridyl)-Tetrazol wird als Folge seiner Stoffwechselbeständigkeit aufgefasst.

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Medical Research Laboratories, Chas. Pfizer & Co. Inc., Groton (Connecticut, USA), 18th July 1966.

[10] V. P. DOLE, J. clin. Invest. *35*, 150 (1956).

[11] Dr. M. SCHACH VON WITTENAU of these laboratories has shown that I is excreted by the dog essentially unchanged over a 24 h period.