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Effect of Tobacco Smoke Condensates on Ascorbate

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INTRODUCTION

Considerable interest exists in the reduction-oxidation properties of tobacco smoke condensates as is evident from several recent publications (1, 2, 8, 9) on this subject, including one from this laboratory which describes the differentiation of different tobacco smoke condensates making use of their dye-reducing property (4). Recently we have become interested in the reaction of smoke with ascorbic acid because of the essential nature of this vitamin for a variety of body functions (10) and the recent suggestion by *Edgar* (6, 7) that dehydroascorbate may be the inhibitor substance "retine" which *Szent-Györgyi* believes is involved, along with another substance, "promine", in controlling cell division (11, 12).

There are several studies of the relationship between smoking and ascorbate levels in various tissues of the body, but, to our knowledge, only that of *Calder* et al. (5) deals with the *in vitro* reaction between smoke and ascorbate. They have observed *in vitro* destruction of ascorbic acid by tobacco smoke but have given no details as to how they studied this reaction. *Taber* and *Larson* (13) while studying the effect of nicotine on ascorbate in aqueous solution and in whole blood, concluded that the destruction of ascorbate was the result of a change in pH brought about by nicotine and not due to a chemical reaction involving nicotine. In this communication we shall show that tobacco smoke condensates markedly accelerate the oxidation of ascorbate and that this property can be used to differentiate smoke condensates from a variety of tobacco types.

MATERIALS AND METHODS

Standard smoking procedures (35 ml puffs of 2-second duration every minute) were employed. When different types of tobacco were smoked in a common vehicle, the cigarette form was used. Smoke solutions were prepared as described previously (4).

A polarographic technique was employed in our studies of the reaction of smoke with ascorbate using a Yellow Springs Instrument Co. biological oxygen monitor (with a *Clark*-type membrane covered polarographic electrode) to measure oxygen uptake. The method consisted of air-saturating 2.8 ml 0.05 M Tris-(hydroxymethyl)-aminomethane-HCl (Tris-HCl) buffer, pH 8.0, by stirring 3 minutes in the glass chamber of the monitor bath assembly, adding 0.1 ml of the smoke solution in ethanol, stirring another 1/2 minute, and initiating the reaction by adding 0.1 ml of the 0.5 M ascorbate solution. The probe was inserted into the chamber, and oxygen uptake recorded. The oxygen concentration before initiating the reaction was taken to be 0.711 µM in 3 ml. Tangents to the curve drawn at the initial rapid rate were used to calculate reaction rates which are expressed as µM oxygen taken up per minute. Specific activity was expressed as µM oxygen uptake per minute per mg TPM*. Appropriate controls to account for the autooxidation of ascorbate were always included. No oxygen uptake was observed by either smoke solutions or tobacco extracts under the conditions of our experiments.

RESULTS AND DISCUSSION

Autooxidation of ascorbate is appreciable, particularly at higher pH. Table 1 shows the rate of oxygen uptake by a 3.3×10^{-3} M solution of ascorbate for the pH range 6.0 to 10.0. Also shown is the oxygen uptake by a similar ascorbate solution over the same pH range in the presence of smoke condensate preparation. On the basis of these figures, pH 8.0 was chosen as the most suitable point for studying the acceleration of oxidation by smoke.

Table 1. Effect of pH on the rate of ascorbate oxidation by cigarette smoke condensate.

pH of reaction mixture (in Tris-HCl buffer)	Rate of ascorbate oxidation mµM O_2 uptake per minute	
	Without smoke	With smoke
6.0	0.0	0.0
7.0	0.0	7.8
8.0	14.2	133.7
9.0	41.2	174.9
10.0	430.2	942.8

* Received for publication: 16th February, 1971.

* Total particulate matter

Table 2. Extraction and stability of ascorbate oxidising activity from cigarette smoke condensates.

Extraction in	Weight of TPM (mg) from 5 cigarettes	Ascorbate oxidation specific activity μM O_2 uptake per minute per mg TPM	
		Initial values	After 18 hours at 20–22° C
0.01 M PO_4 buffer, pH 5.7	121.9	0.22	0.09
do. pH 6.5	120.7	0.18	0.04
do. pH 7.0	125.1	0.11	0.02
do. pH 7.5	123.8	0.08	0.02
do. pH 8.0	125.9	0.07	0.02
Ethanol, pH 6.1	128.6	0.65	0.41

To optimise removal from the filter pads of the smoke components responsible for ascorbate oxidation, several extraction conditions were tested. In Table 2 are shown the efficiencies of extraction and stability of the extract of a number of 0.01 M phosphate buffers of varying pH and also 95% ethanol. Increasing acidity improves the extraction efficiency of the buffers but the oxidising activity is unstable in solutions of this type. Ethanol is a better solvent than buffer solutions with respect to yield and stability of oxidising activity. Similar results were obtained for extraction of dye-reducing activity from smoke condensates (4). No oxidising activity was found in the vapour phase of cigarette or pipe smoke; small amounts exist in cigar smoke vapour phase.

Ascorbate Oxidising Activity in Cigarette, Cigar and Pipe Tobacco Smoke Condensates

To facilitate intercomparison of the activities of particulate matter from the different smoking vehicles, experiments were carried out on TPM dried at 4° C in a desiccator. This drying procedure was found to be necessary on account of the large amounts of water accumulating on the Cambridge filter during pipe-smoking. It was found that smoke from flue-cured cigarettes had the highest activity (Table 3). It is not possible to differentiate between brands containing this type of tobacco and filters do not affect the specific oxidising activity of the condensates. Blended cigarettes, containing other tobacco types in addition to flue-cured, occupy an intermediate position while cigar and pipe smoke TPM have activities of a lower order and are similar to each other.

Table 3. Ascorbate oxidation by tobacco smoke condensates.

Condensate from	Ascorbate oxidation specific activity μM O_2 uptake per minute per mg TPM	Condensate from	Ascorbate oxidation specific activity μM O_2 uptake per minute per mg TPM
CIGARETTES		CIGARS	
Flue-cured		Brand 1	0.16
Brand 1	0.52	2	0.09
2	0.50	3	0.13
3	0.58	4	0.14
4	0.53		
Blended		PIPE-SMOKING	
Brand 5	0.28	Brand 1	0.12
6	0.37	2	0.17

Ascorbate Oxidising Activities of Tobacco Types Smoked in Cigarette Form

Since differences were observed between cigarette, cigar and pipe smoking with respect to ascorbate-oxidising activities, it was considered important to find out how the tobaccos would behave if smoked in a common vehicle. Pipes could have been used for this purpose, but on account of the variation observed in pipe-smoking, they were regarded as unsatisfactory. Consequently, three types of tobacco, flue-cured, air-cured and burley, also cut and rolled flue-cured stem, and reconstituted tobacco were smoked in cigarette form and tested for ascorbate-oxidising activities. The results obtained are presented in Table 4. As expected, the flue-cured tobacco and 100% flue-cured stem gave smoke condensates with higher activities than those from air-cured, burley or reconstituted tobacco.

Table 4. Redox properties of tobacco types smoked in cigarette form.

Tobacco type	Ascorbate oxidation specific activity μM O_2 uptake per minute per mg TPM
Flue-cured	0.58
Air-cured (cigar tobacco)	0.17
Air-cured (burley)	0.25
100% reconstituted	0.41
100% flue-cured stem	0.67

Ascorbate Oxidising Activities of Tobacco Types

It was previously found that no significant quantities of dye-reducing activity existed in tobacco or ash (4). Ascorbate oxidising activity was, however, present in both. Ten grams of flue-cured tobacco were soaked overnight in either 95% ethanol, water or 0.01 M phosphate buffers ranging from pH 5.7 to 8.0. The

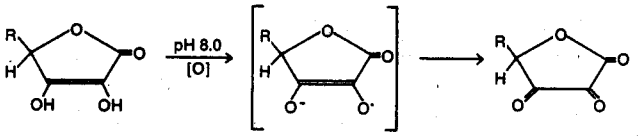
Table 5. Ascorbate oxidising activity of cigarette tobacco extracts.

Extraction in	Ascorbate oxidation μM O_2 uptake per minute per gram tobacco
0.05 M PO_4 buffer, pH 5.7	10.23
do. pH 6.0	8.46
do. pH 6.5	9.60
do. pH 7.0	8.03
do. pH 7.5	8.67
do. pH 8.0	8.53
Water	6.68
Ethanol	2.77

oxidising activities of the extracts are expressed in Table 5. The most active extract was obtained with buffer at pH 5.7, while the least efficient solvent was ethanol, suggesting that the compounds in tobacco with oxidising activity are different from those in TPM. Cigarette ash contained small amounts of this activity, while ashed TPM retained 10% of the activity of the TPM.

Mechanism of Ascorbate Oxidation by Smoke

Autooxidation and enzymic oxidations of ascorbate are known to proceed through a free radical intermediate (3, 14, 15). The reaction, under our experimental conditions can be represented by Equation 1.

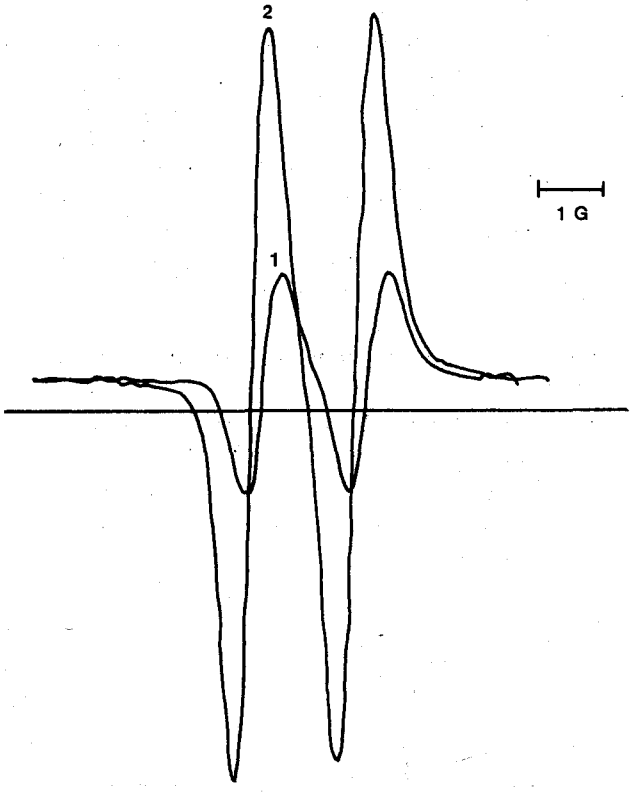


To verify that smoke oxidation and autooxidation have a common mechanism, EPR studies were made on both reactions, using a Varian E 3 EPR spectrometer and a flow cell connected to a metering pump. The signal due to the radicals formed during autooxidation was first measured; then an aqueous smoke solution at the same pH was introduced and the spectrum was again scanned. The results are shown in Figure 1. The increase in signal intensity indicates that the smoke accelerated reaction does in fact proceed through the same radical intermediate as shown in Equation 1.

As mentioned earlier, aqueous tobacco extracts have ascorbate oxidising ability. This oxidation was also found, by a similar EPR examination, to involve the same intermediate.

Figure 1. EPR spectra of ascorbic acid free radical formed during continuous flow [1] autooxidation and [2] smoke accelerated oxidation.

Medium: aqueous-7% ethanol, pH 8.0.
g: 2.004.



SUMMARY

Ethanolic extracts of tobacco and tobacco smoke contain compounds capable of accelerating the oxidation of ascorbate. Using a polarographic technique, smoke from cigarettes, cigars and pipe were examined for oxidising properties. Smoke from Virginia cigarettes showed greater activity than that from blended cigarettes, while smoke from cigars and pipes had even lower activity.

The reaction of ascorbate and smoke has been shown by EPR to involve a radical intermediate.

ZUSAMMENFASSUNG

Äthylalkohol-Extrakte von Tabak und Tabakrauch enthalten Verbindungen, die die Oxydation von Ascorbaten beschleunigen können. Diese oxydierenden Eigenschaften des Rauches von Cigaretten, Zigarren und Pfeifen wurden mittels einer polarographischen Methode untersucht. Der Rauch von Virginia-Cigaretten zeigte eine größere Aktivität als der von Blend-Cigaretten, während die Aktivität von Zigarren- und Pfeifenrauch noch geringer war.

Es wurde durch EPR nachgewiesen, daß die Reaktion von Ascorbat und Rauch über die Zwischenstufe eines freien Radikals verläuft.

RESUME

Les extraits éthanoliques du tabac et de la fumée de tabac contiennent des composés capables d'accélérer l'oxydation de l'acide ascorbique. Se servant d'une méthode polarographique, la fumée de cigarettes, de cigars et de tabac à pipe a été étudiée en rapport avec ses propriétés oxydantes. La fumée provenant de cigarettes à tabac de Virginie montra une plus grande activité que celle provenant de cigarettes contenant d'autres types de tabac ajoutés au tabac jaune, tandis que la fumée provenant de cigars et de tabac à pipe avait une activité plus basse.

La réaction de l'acide ascorbique et de la fumée, illustrée par EPR, implique un radical intermédiaire.

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