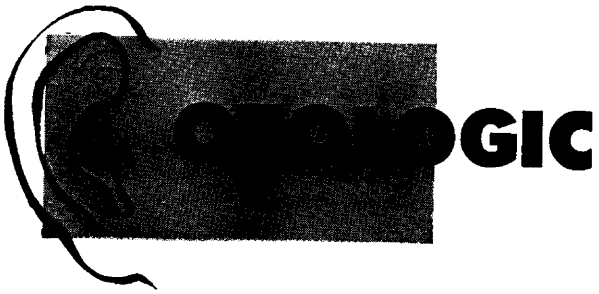

Experimental Tobacco Poisoning

Resultant Structural Modifications of the Cochlea and Tuba Acustica

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The negative effect of tobacco on the human body has already been extensively demonstrated. Information of general and specialized interest [1-4] and historical notes on this subject [4] have already been mentioned in our previous articles.

Bibliographic Information

Many studies have been published with regard to the action of tobacco smoke on the human organism, and the bibliography has already been reported by us elsewhere. We here merely recall some very recent articles [5,6] and reports of medical congresses [7] illustrating the great interest of modern research workers in this subject.

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Damage to the hearing function in tobacco smokers has been reported from as far back as the middle of the nineteenth century and from the very first years of the present century.[8-11] As regards the symptoms, general hearing disturbances [12-14] have been observed along with deficient hearing, buzzing in the ears,[15] and a distinctly perceptive deficit with buzzing of the ears and vertigo [16,17]; deficiency of perceptive type with shortening of the perception time of the osseous route [18]; deficit of mainly cochlear type or mainly vestibular type (or both combined) with labyrinthine excitability which is found to be distinctly outside normal limits on routine tests [19,20]; Ménière-like syndromes [21,22]; and isolated, not very serious labyrinthine disturbances [23,24] including some with the presence of spontaneous nystagmus.[25]

Of these various authors—almost all of whom date back some time—mention must be made of Tempea,[26] who considered a toxic neuritis of the VIII pair of cranial nerves, and of Brunner,[27] who attributed a special toxicity for the vestibular system to nicotine. Delie[17] believed that the vertigo and buzzing of the ears observable in subjects intoxicated by tobacco smoke were to be attributed to anemia of the semicircular canals and to a circulatory deficit (sometimes irreversible) of the cochlea. Very schematically, this author attempted to distinguish between: (*a*) an infrequent degeneration of the acoustic nerve due to selective toxicity of the components of tobacco smoke on this nerve, and (*b*) a regional vasospastic circulatory action terminating in a state of trophoneurosis. According to this author, all cases of recovery following suppression of the use of tobacco should etiologically be attributed to the second type of lesion.

The opinions expressed by Delie[17] were almost entirely confirmed by other authors,[28,29] while some other investigators[30] believed that the etiology of the various states of deficient hearing observed in smokers could be attributed to the frequent forms of catarrhal salpingitis observed in such subjects.

In contrast, Van Caneghem[31] has stated that the alterations of the acoustic and vestibular functions in heavy smokers are attributable to a lesion of the endolabyrinthine circulatory system (of hypertensive type) conditioned by the vascular alterations produced by the components of tobacco smoke.

As regards deficient hearing and vertigo sometimes observed in workers engaged in the manufacture and preparation of tobacco, readers are referred to the literature already quoted by us in a previous article.[2] The same holds true for the relationship between optical neuritis and optical disturbances and vestibular disturbances sometimes clinically observable in the same patient intoxicated by the use of tobacco.

Some authors have experimentally studied the action of the components of tobacco smoke on various types of animals. The techniques used for these studies are, however, very different from that used by us (these authors generally injected nicotine parenterally). Gradual chromatolysis and plasmolysis of the ganglionic cells,[32] degeneration of wallerian type (with chromatolysis, dendritic atrophy, and formation of vacuoles),[34] cellular alterations of spiral ganglion and cerebral cells,[35] lesions of the nuclei of the cells in the vestibular ganglion,[36] and alterations of the nervous elements probably depending on the primitive alterations of the vascular system[37] have all been observed.

Personal Researches

Our researches were carried out experimentally on guinea pigs.

In order to be able to observe the action of smoke on the organs and apparatus which it was our intention to study, we considered it opportune to administer to the animals tobacco smoke obtained under conditions which would not make it very different from the smoke which is obtained when tobacco is burned and inhaled by a normal smoker. Furthermore, it seemed expedient not to resort to any operation on the animals to introduce the smoke directly into the respiratory apparatus (on the other hand, this solution has already been adopted by other authors such as Vincent, Segonzac, and Lagreu,[38] Jourdan and Collet,[39,40] and others) nor to oblige the animals to adopt forced positions for a long time during the experiment but rather to keep them under the most suitable conditions for the purposes of our research.

Before outlining our simple technique, we should like to remind the reader of the methods adopted by some other authors among those who, before us, have administered tobacco smoke directly to the animals for a longer time and with different methods. Roffo,[41] in an attempt to produce leukoplakic lesions, blew tobacco smoke onto

the oral mucosa of the rabbit for 5 minutes a day and for periods of time not longer than a few weeks. Lorenz, Stewart, Daniel, and Nelson [42] exposed rats to tobacco smoke for a period varying from 25-250 days; they used a special apparatus by means of which the rats were subjected to a maximum of exposure of 693 hours; the average smoke content of the experimental surrounding air was about 1 mg. per liter. More recently, Kreshover,[43] in order to study the carcinogenetic effect of tobacco, devised an apparatus, fully illustrated in his paper, by means of which one could put tobacco smoke into contact with the lips and ears of the rats undergoing the experiment: the procedure was such as to permit the animals to receive equal applications according to number, time of exposure, periods of interval, and volume of smoke.

It is almost superfluous to add that the methods mentioned up to now, and other similar methods applied by other authors, did not appear to be suitable for our experimentation, mostly because of the different topography of the organs studied by us in comparison with the parts of the body investigated by the above-mentioned authors.

At this stage, we ought to point out that some authors [44] have advanced the hypothesis that tobacco smoke, administered to animals in an atmosphere filled with the smoke itself, is not equal to that which smokers usually inhale: this is said to occur through the relative physical and chemical instability of the smoke itself.

We, on the contrary, and other authors with us, have considered valid the method which takes advantage of the intoxication of the animal by means of smoke administered in the atmosphere: this is both because by these methods the smoke is administered repeatedly at regular intervals with a continually renewed supply of smoke which is immediately inhaled, and also because the necessity for studying the toxicology of tobacco smoke in all its components renders this method and the way of inhalation preferable to any other quite abnormal method of administration, the possible consequence of which could be that the components of the smoke may be still more elaborated and in a condition which might bring about a different metabolization on the part of the organism. In fact, it is evident that the effects produced by the pyridinic bases, by the alkaloids, by the ethereal oils, etc., can never, separately, be compared with the physical and chemical effects which smoke is capable of provoking in its entirety.

Our technique of intoxication of the animals has, on the other hand, been regarded as valid by other authors who have concerned themselves with this subject and who have applied methods at least partly comparable to ours. Thus Essenberg,[45,46] in order to study the behavior of some glands toward internal secretion and the insurgency of pulmonary carcinoma in rats, placed the animals in a cabinet 2 cubic feet in volume, filled automatically with smoke obtained from cigarettes, for 12 hours a day, every day except Sunday; each cigarette was burned in about 4 minutes, and for a few minutes after the end of the combustion of the tobacco the atmosphere enclosing the animals remained almost saturated with smoke, after which a stream of fresh air was made to pass through it. Essenberg obtained the smoke by producing a slight depression in the animals' chamber; this depression attracted into the chamber itself cigarette smoke which was supplied from time to time through a hole drilled in one of the walls.

For our researches we found it necessary to utilize methods of tobacco intoxication similar, at least in part, to those used by other authors such as Essenberg, for example. The animals were placed in a zinc container with a floor area of 90×60 cm. and 50 cm. in height (2 of these containers were prepared, in view of the number of animals undergoing the experiments daily) with 2 glass walls for observation of the animals. In the 2 smaller walls, 2 holes of about 3 cm. in diameter were bored.

A hand pump was connected by means of a very short rubber tube and 2 ducts. One

of the ducts was in the shape of a cigarette-holder, and a lighted cigarette was applied to its extremity.

With a system of Mohr tweezers, smoke was drawn from the lighted cigarette by each suction movement of the pump; when the tweezers belonging to the duct connected to the cigarette-holder was closed, the second duct opened, and the smoke was forced through it into the cabinet through 1 of the 2 holes. This maneuver, being very simple, could be carried out very rapidly so as to ensure that the smoke did not remain in the tubes for more than a very short time. Four guinea pigs were placed in the container each time. The temperature of the experimental atmosphere was constantly watched and never underwent abnormal rises. This may also be said of the carbonic anhydride which was absorbed by means of a convenient quantity of calcium soda; nor was the water vapor ever excessive. From both holes drilled in the cabinet (when the first was not occupied by one of the ducts belonging to the apparatus for the production of smoke) atmospheric air flowed in sufficient quantities: the proof of this lies in the fact that the animals kept in the experimental atmosphere for long periods of time did not show any sign of suffering when tobacco smoke was not being injected into it.

The smoke produced by the various cigarettes, being introduced into the cabinet, made its atmosphere very rich and obliged the guinea pigs to take forced inhalation of tobacco smoke.

Of course, the inhalation of the smoke, when the smoke itself was present in the cabinet, was continuous instead of at more or less regular intervals as in the case of smokers; but we were careful to study every means of making the smoke as far as possible equal in composition to that which is normally obtained from cigarette smoke immediately it is inhaled. For this reason, we took care to use particularly short tubes for the introduction of the smoke by and through the little pump used in the device in view of the strong tendency of smoke to deposit the substances present in it. This deposit of residue seemed to us to be directly proportional to the length of the tubes, to the slowness of the passage of the smoke through the tubes, to the cooling of the smoke, and to the speed of combustion of the cigarette. With reference to this latter factor, we always pumped the smoke with suction movements spaced out by long pauses in such a way as to burn a cigarette in about 3-9 minutes, which is the time normally taken by a man to smoke one. In this way, the smoke did not issue from combustion at an excessively high temperature, as would have been the case, on the contrary, if combustion had been continuous; we were thus enabled to inject the smoke with greater regularity into the cabinet containing the animals.

With this method we kept, as may be observed, only partly to the systems adopted by other authors as, for example, Essenberg.[45,46] Unlike this author, we made special efforts to avoid any factor which could induce abnormal variations in the atmosphere in which the guinea pigs had to stay, except, of course, for the presence of the smoke.

For this reason, we did not produce a depression in order to attract the smoke into the container, thinking that this factor might have disturbed the animals, if only slightly.

In addition, we kept the animals in the experimental atmosphere only at intervals for the time that was necessary (not for very long periods, even during the intervals of rest, as other authors have done), since we observed that the walls of the cabinet become impregnated, after several months of exposure to smoke, with the products of distillation of the smoke itself, thus leading to the discomfort of the animals being experimented upon, through causes which destroy the finality of our researches.

We used the type of cigarette labeled "Nazionali"; this choice was motivated by the fact that we chose to study the toxic effects provoked by a type of tobacco consumed very widely in Italy.

Three groups of animals were studied (each group comprising 4 guinea pigs).

The first group was subjected to tobacco smoke poisoning with massive doses of smoke administered in a short period (the animals lived for a time varying from 18-55 hours; the quantity of smoke used in the experiment was produced by a number of cigarettes varying from 22-65 for each animal. The guinea pigs were allowed to rest for half an hour in fresh air every hour).

The second group was subjected to tobacco smoke poisoning for a period of 2 months (the animals were exposed to tobacco smoke for 2 hours in the morning and 2 hours in the evening, a total of 8 cigarettes being burnt daily. Progressively increasing minimum doses were used at first).

The third group was subjected to tobacco smoke poisoning for a period of 17 months (the animals were gradually habituated to support the smoke supplied by a quantity of cigarettes varying from 7-12 daily; exposure to the smoke lasted for approximately 5 hours subdivided into 2 or more periods during the day).

It appears clear from the above that we wished to study the histologically observable experimental modification of the cochlea and of the mucosa of the eustachian tubes in animals subjected to the action of cigarette smoke and treated acutely (Group 1), subacutely (Group 2), and chronically (Group 3).

The animals were killed by injection of air into the heart. Histological preparations were obtained by fixing in formalin or else in Bouin's fluid or Carnoy A. The material was always studied on sections made in series, and the staining was as follows according to the case: Lhermitte or toluidine blue for the tigroid bodies; or else hematoxylin and eosin; Nissl's method for neural tissue and for chromophil substances; cresyl violet; or finally, Van Gieson's stain.

Cochlear Findings

Group 1.—The vascular alterations observed in this group were marked. Apart from vasal thrombosis (Fig. 1) which can be observed in some sections, the endothelium of the intima becomes particularly evident with a distinct showing-up of the nuclei of these vascular sheathing cells, a finding which could be attributed to subintimal edema linked to the action of tobacco smoke. The vascular lacunae of the stria vascularis appear normal or slightly dilated.

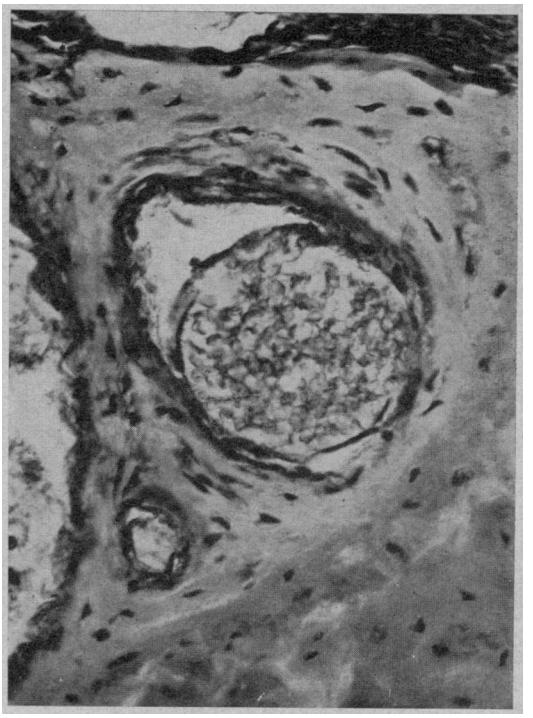


Fig. 1.—Thrombosis of the artery of the basal cochlear whirl (acutely intoxicated guinea pig). Fixing in Bouin's liquid; staining: hematoxylin and eosin.

A few hemorrhages were observed in the perilymphatic space, probably due to the intense cochlear vascular upheaval arising acutely in the animals of this group.

We also observed mild hypertension in the perilymphatic states; however, it was impossible to exclude the possibility that this was an artifact due to the technique employed.

Corti's organ and the acoustic nerve showed nothing frankly pathological. The spiral ganglion also appeared to be generally in good condition; only mild intercellular edema was observed (Fig. 2).

Our histological observations were extended not just to the cochlear but also to

the encephalon of the intoxicated guinea pigs. Animals of the group which had acutely inhaled the large amount of tobacco-smoke revealed degenerative factors of marked degree involving the cerebral matter (Fig. 3) with cellular vacuolization affecting the nerve tissue. This finding is present to a much slighter degree in animals of the other 2 groups who gradually became habituated to the poisons administered.

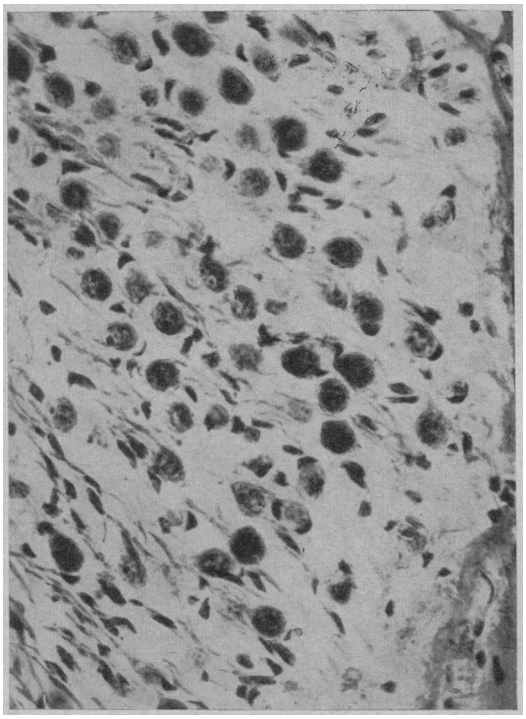


Fig. 2.—Initial intercellular edema in elements of Corti's ganglion of acutely intoxicated guinea pigs. Fixing in Bouin's liquid; staining: hematoxylin and eosin.

Group 2.—The very interesting finding in this group is in relation to Corti's ganglia. These reveal, although not in all animals, degenerative factors with mild rarefaction of the ganglial cells. Apart from the presence of intercellular edema, it is sometimes possible to observe a slight increase of the connective tissue present between the ganglial cells (Fig. 4).

Hemorrhages are frequently observed, particularly at the level of the stria vascularis in its lower portion. This point of origin of the hematic extravasations justifies

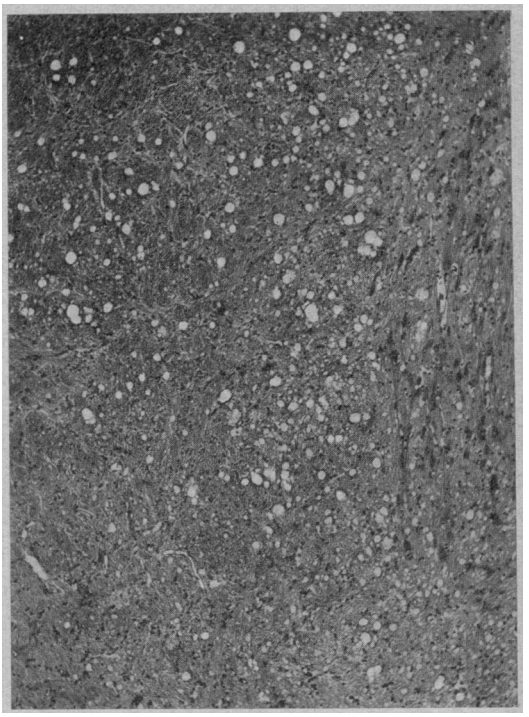
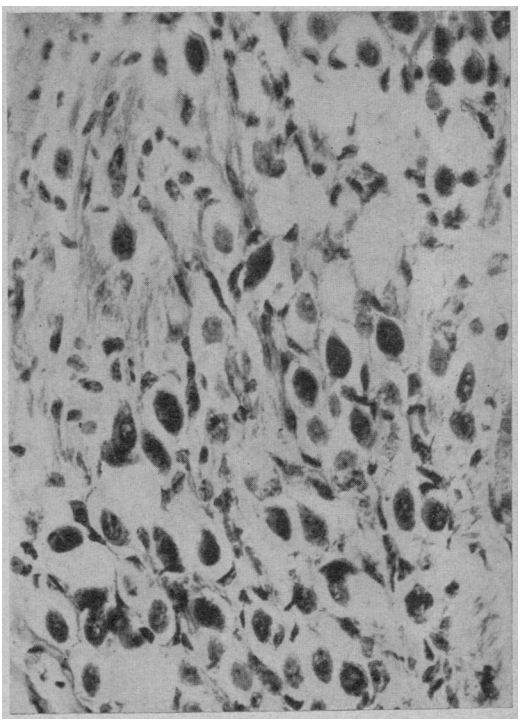


Fig. 3.—Degenerative phenomena involving the cerebral matter of acutely intoxicated guinea pigs. Fixing in formalin; staining: Nissl's method.

Fig. 4.—Subacutely intoxicated animals may reveal a slight increase of the connective tissue between the cells of Corti's ganglion. Fixing in Bouin's liquid; staining: hematoxylin and eosin.



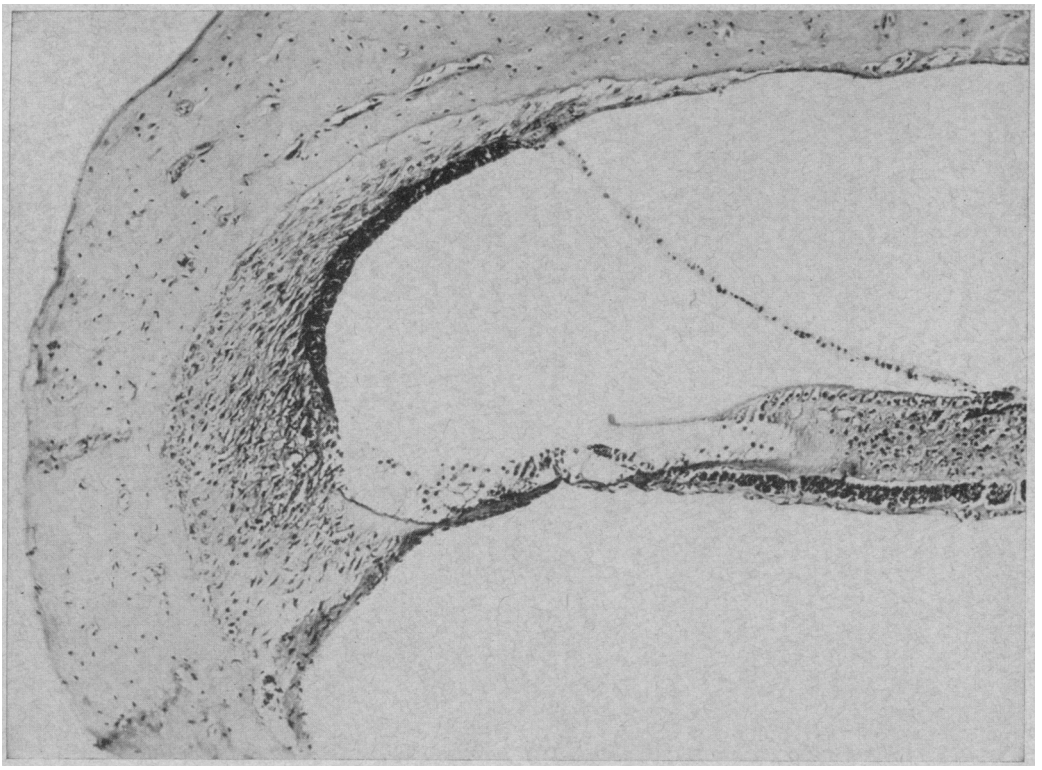


Fig. 5.—Initial cytoplasmic degeneration of the cellular elements of Corti's organ in the cochlea of a subacutely intoxicated guinea pig. Fixing in Bouin's liquid; staining: hematoxylin and eosin.

the presence of abundant blood cells at the site of the scala tympani. We sometimes observed vacuolization and cytoplasmic swelling in Corti's organ, with cells revealing indistinct and badly distinguishable outlines (Fig. 5).

As regards the presence of a pressure preponderance of the endolymphatic liquids, sometimes revealed by the position assumed by Reissner's membrane, we are of the opinion that the histological method is not the most suitable for ascertaining the true existence of this, and we cannot therefore exclude the possibility that a technical artifact is involved.

Group 3.—The vascular symptoms observed in the previous groups were here less intense, although present. They were most obvious in the perineural zones.

The limits of the stria vascularis appeared to be especially altered, revealing many blood cells oozing out from the vasal lacunae of these formations due to diapedesis. Intense degenerative symptoms were present, involving the cells of Corti's organ (Fig. 6). Reissner's membrane was generally situated in such a position as to justify eventual hypertension of the endolymphatic fluids, as has already been hypothetically admitted by some previous authors.[31]

Nothing pathological involved the acoustic nerve, while in contrast, the spiral ganglion revealed a reduced number of cells separated from each other by obvious intercellular edema. Degenerative signs, both protoplasmic and nuclear (Figs. 7 and 8), were revealed in these.

Eustachian Tube

As previously observed, the hypothesis suggested by some authors[30] relative to an etiology of the loss of hearing due to tobacco, which is based on catarrhal salpingitis caused by the components of the smoke, does not appear completely unjustifiable.

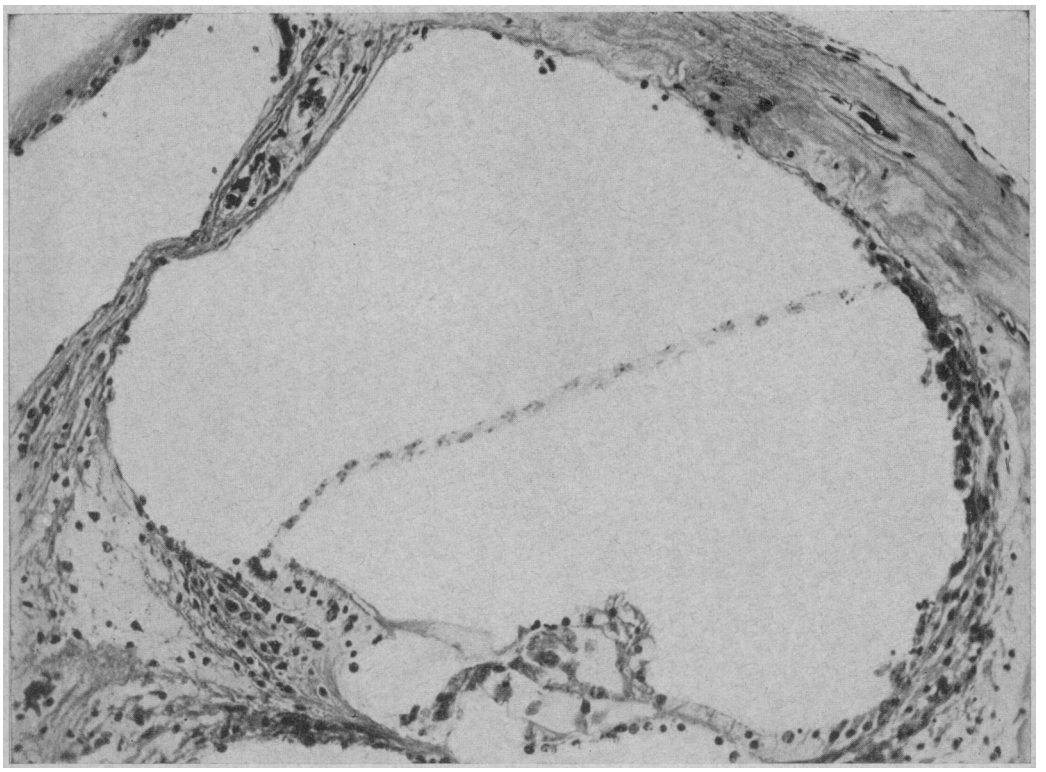


Fig. 6.—Cochlea of chronically intoxicated animal. Corti's organ appears to be degenerated; the limits of the stria vascularis appear ill-defined because of the hemorrhages resulting from diapedesis and from degenerative manifestations. Fixing in Bouin's liquid; staining: hematoxylin and eosin.

The observation of the eustachian tube in the animals we studied revealed the following:

Group 1: normal tubal epithelium;

Group 2: presence of abundant exudate in the lumen of the tube and mild initial involvement of the epithelium of the salpinges (Fig. 9);

Group 3: marked involvement of the tubal mucosae, poor secreting, with flattened cells poor in cilia, and with submucosa reduced in thickness and poorly supplied with blood. It may be stated in these cases that tobacco smoke has provoked mucosal atrophy (Fig. 10).

Conclusions

From the clinical point of view, many authors, including one of us,[47,48] have already noted the harm brought by tobacco both to the cochlear-vestibular apparatus in isolation and to this apparatus and the retina together. The present studies were mainly directed at understanding which of the etiological hypotheses suggested in the past was the most probable and which, furthermore, were the particular structures of the auditive system most affected.

The cochlear damage appears to be attributable to a primitive alteration of the vascular structures of hearing. Chronically intoxicated animals always showed degeneration of the neurosensorial epithelia and of some specific cochlear structures besides the tubal mucosa. Less important, or at least less detectable, appear to be the pressure alterations of the labyrinthine fluids.

In conclusion, we feel that 2 types of lesions can be observed: (*a*) lesions of a vascular nature observable in acutely intoxicated animals; and (*b*) degenerative lesions of the noble elements of the cochlea, overlapping and consequent to the vascular lesion, which are always present and observable in chronically intoxicated animals. (Subacute type lesions may be classified as

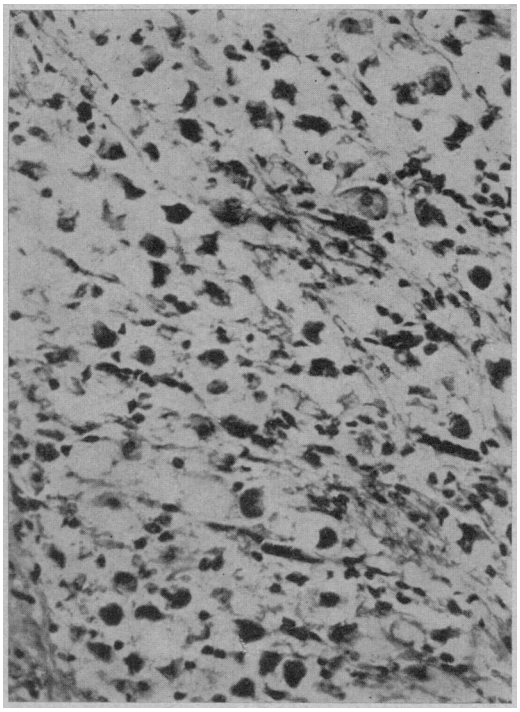


Fig. 7.—Obvious degeneration of Corti's ganglion in chronically intoxicated guinea pig. Fixing in Bouin's liquid; staining: hematoxylin and eosin.

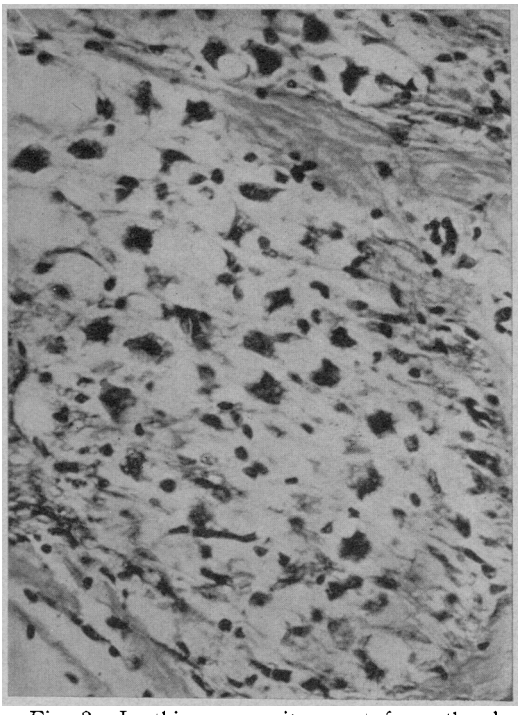


Fig. 8.—In this case, quite apart from the degenerative manifestations observed in the previous figures, there is also an advanced state of intercellular edema. Staining and fixing as in the preparation shown in the previous figure.

Fig. 9.—Presence of abundant exudate in the lumen of the eustachian tube of a subacutely intoxicated guinea pig. Fixing in formalin; staining: hematoxylin and eosin.

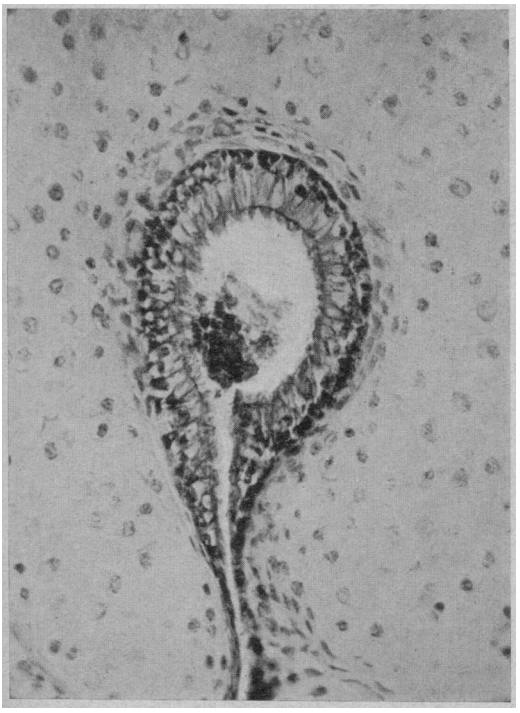
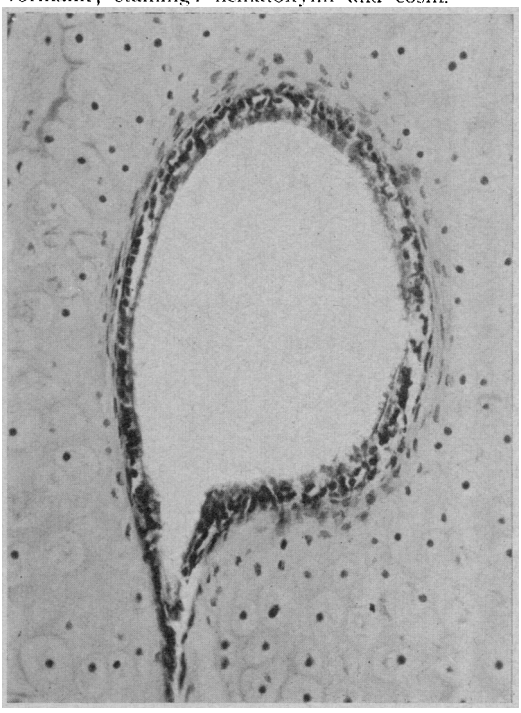


Fig. 10.—Absence of exudate, atrophy of the mucosa, and loss of the vibratile cilia in the tube of a chronically intoxicated guinea pig. Fixing in formalin; staining: hematoxylin and eosin.



very similar to those of frankly chronic type.)

Furthermore, the lesions of the eustachian tubes contribute to the acoustic damage in subjects already affected by the marked and severe cochlear lesions previously revealed.

In recognizing this as the mechanism in the hypoacousis provoked by the action of tobacco smoke, we have partly associated ourselves with the views of Delie,[17] Bouchet and Labayle,[28] Hohlbrugger,[29] and other research workers, and also with those of Beynes,[30] but it seems more difficult to us, at least in the experimental field, to adhere to the etiopathogenetic hypotheses of Van Caneghem,[31] who attributes the cochlear lesions mostly to alterations of the tension of the labyrinthine liquids. In effect, we, like Van Caneghem,[31] think that the first etiopathogenetic moment of the cochlear lesions is conditioned by primitive vascular alterations induced by the smoke; however, as has in part been shown by our observations, it is not always possible to affirm that these vascular alterations promote constant and conspicuous variations of the pressure of the endolabyrinthine liquids, while sometimes—still in the absence of pressure variations—degeneration of the elevated epithelium and of other elements of the cochlea is clearly to be observed, to all appearances induced by the same vascular variations as those mentioned above.

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