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(1) From the University Institute for Cancer Research in Buenos Aires. Director: Professor A. H. Roffo

(2) Tobacco as a Carcinogenic Agent
by Prof. A. H. Roffo

(3) For a long time much has been written about the effect of tobacco on the occurrence of cancer. (4) In hospitals, a connection has been observed between the frequently found lip cancer and pipe smoking. (5) However, these were only experiments which needed to be proven by experiments. (6) The experiments we conducted not only demonstrated the dangers to which the smoker is exposed, but also the role of tobacco as a carcinogenic agent. (7) They also show to which of the different tobacco combustion products the actual etiological property can be attributed.

(8) As early as 1928, when I was working with the patients examined and treated during the out-patient consultation hours (I), I pointed out the large number of patients with cancer of the mouth or throat, who were all heavy smokers. (9) When I analyzed this material statistically, I noticed another interesting fact regarding the classification of the patients according to sex, inasmuch as females very rarely contract these forms of cancer. (10) The observations I made over 12 years on the few women with cancer in these areas are of some experimental value, since all of these women were heavy smokers. (11) During the time period mentioned, I observed a total of 5,000 women who had cancer, among whom were only 42 cases with cancer in the so-called "smoke path", but all of the 42 were heavy smokers. (12) In certain years, for example in 1932, not a single woman with cancer of the throat came for treatment.

(13) Carcinomas of the so-called smoke path in woman have been localized mainly on the lip, tongue, and larynx, which can be seen from the following table:

lips	28.95%
tongue	28.45%
larynx	18.47%
soft palate	14.48%
pharynx	9.65%

(14) A summary of the medical histories of the women with cancer in the so-called smoke path is as follows.

1. Patient number 24597, M. G. D., Argentine, 69 years old. Heavy cigar smoker. Tumor of the lower lip and the rim of the mouth. Biopsy: surface carcinoma with beaded formations.
2. Patient number 13607, C. F., Argentine, 43 years old. Cigar smoker. Vegetative carcinoma on the corner of the mouth and leukoplasia of the mouth. Biopsy: surface carcinoma.
3. Patient number 21109, P. A. K., Argentine, 64 years old. Cigarette smoker, 1 1/2 packs per day. Carcinoma of the tongue with ganglia of the throat. Biopsy: metatypical surface carcinoma.

4. Patient number 21014, A. V., Argentine, 65 years old. Heavy cigar smoker. Carcinoma of the base of the tongue. Biopsy: metatypical surface carcinoma.

5. Patient number 29957, L. S. S., Arab, 66 years old. Heavy cigarette smoker, 2 packs per day. Carcinoma of the right rim of the tongue.

6. Patient number 23343, A. F. F., Argentine, 42 years old. Heavy cigarette smoker, 2 packs per day. Carcinoma of the tongue.

7. Patient number 24806, A. A. S., 44 years old. Heavy cigar smoker, 10 cigars per day. Carcinoma of the pharynx including the [illegible].

8. Patient number 2715, C. A. V., Argentine, 58 years old. Heavy cigar smoker, 12 to 15 cigars per day. Carcinoma of the bottom of the mouth. Biopsy: surface carcinoma.

9. Patient number 10131, N. C., Argentine, 40 years old. Heavy cigar smoker, 10 to 12 cigars per day. Biopsy: surface carcinoma.

10. Patient number 11895, M. A. A., Turkish, 21 years old. Heavy black cigarette smoker. Cancer of the base of the tongue. Biopsy: surface carcinoma with beaded formations.

11. Patient number 16764, F. V. D., Argentine, 69 years old. Heavy cigarette smoker. Cancer of the bottom of the mouth. Biopsy: basophilic carcinoma.

12. Patient number 18030, F. E., Argentine, 56 years old. Pipe smoker, 6-8 per day. Carcinoma of the soft palate and the cheek. Biopsy: metatypical surface carcinoma.

13. Patient number 25619, A. L., Swiss, 58 years old. Black cigarette smoker. Carcinoma of the soft palate. Biopsy: metatypical surface carcinoma.

14. Patient number 3040, F. T. R., Argentine, 60 years old. Heavy cigar smoker. Carcinoma of the tongue.

15. Patient number 18169, E. C. R., Italian, 46 years old. Heavy pipe smoker. Carcinoma of the tongue. Biopsy: metatypical surface carcinoma.

16. Patient number 8200, N. P. R., Argentine, 74 years old. Heavy cigar and cigarette smoker. Carcinoma of the lower lip. Biopsy: callous surface carcinoma with beaded formations.

17. Patient number 21934, E. B. B., Italian, 60 years old. Black cigarette smoker, 1 pack per day. Carcinoma of the epiglottis propagating to the tongue and larynx. Biopsy: surface carcinoma.

18. Patient number 21882, M. B. L., Argentine, 33 years old. Heavy cigarette smoker, 2 packs per day. Carcinoma of the pharynx. Biopsy: surface carcinoma with beaded formations.

20. Patient number 17240, N. R. B., Argentine, 68 years old. Heavy cigarette smoker. Surface carcinoma.

21. Patient number 18859, C. B. U., Argentine, 28 years old. Heavy cigar smoker. Carcinoma of the pharynx. Biopsy: surface carcinoma with beaded formations.

22. Patient number 54220, Argentine, 58 years old. Heavy cigarette smoker, 2-3 packs per day. Carcinoma of the base of the tongue. Biopsy: surface carcinoma.

23. Patient number 51150, M. S., Argentine, 65 years old. Heavy [illegible] smoker. Carcinoma of the lower lip with metastases.

24. Patient number 49117, A. P. C., Italian, 72 years old. Pipe smoker. Carcinoma of the lower lip. Biopsy: metatypical surface carcinoma.

25. Patient number 57136, M. C. C., Turkish, 49 years old. Heavy cigarette smoker, 3 packs per day. Carcinoma of the larynx and the base of the tongue. Biopsy: metatypical surface carcinoma.

26. Patient number 28278, M. M., Argentine, 48 years old. Cigar smoker. Carcinoma of the rim of the tongue. Biopsy: metatypical surface carcinoma.

27. Patient number 28725, S. V. C., Argentine, 68 years old. Black cigarette smoker. Carcinoma of the soft palate. Biopsy: metatypical surface carcinoma.

28. Patient number 25565, E. J. P., Spanish, 63 years old. Heavy cigar smoker. Carcinoma of the soft palate. Biopsy: metatypical surface carcinoma.

29. Patient number 31950, M. V. S., Argentine, 79 years old. Heavy cigar smoker. Carcinoma of the tongue. Biopsy: surface carcinoma.

30. Patient number 36496, P. I. A., Argentine, 46 years old. Heavy cigar smoker. Carcinoma of the tongue. Biopsy: metatypical surface carcinoma.

31. Patient number 38955, Argentine, 56 years old. Heavy cigarette smoker, 2 packs per day. Carcinoma of the pharynx. Biopsy: metacypical surface carcinoma.

32. Patient number 43554, A. M. F., Argentine, 79 years old. Heavy cigar smoker. Carcinoma of the lower lip. Biopsy: surface carcinoma.

33. Patient number 44224, M. B. S., Swiss, 82 years old. Heavy pipe smoker. Carcinoma of the lower lip and corner of the mouth.

34. Patient number 46715, M. D. B., Argentine, 63 years old. Heavy pipe smoker. Carcinoma of the upper lip and corner of the mouth.

35. Patient number 49989, G. M. M., Argentine, 84 years old. Heavy pipe smoker. Carcinoma of the tongue. Biopsy: metatypical surface carcinoma.

36. Patient number 50452, C. P. L., Uruguayan, 62 years old. Heavy cigarette smoker. Tumor of the tongue. Biopsy: spindle cell carcinoma.

37. Patient number 53336, L. F. G., Italian, 63 years old. Heavy cigarette smoker. Carcinoma of the lips and metastases of the ganglia.

38. Patient number 52989, F. B. N., Italian, 75 years old. Black cigarette smoker. Carcinoma of the upper lip and corner of the mouth.

39. Patient number 53792, P. R. D., Spanish, 70 years old. Cigarette smoker. Carcinoma of the upper lip and metastases of the ganglia.

40. Patient number 54013, M. A. J., Turkish, 74 years old. Heavy black cigarette smoker. Carcinoma of the lips.

41. Patient number 52530, C. L. H., Argentine, 59 years old. Heavy black cigarette smoker (20-30 daily). Leukoplasia of the mucous membrane of the cheek.

42. Patient number 62980, A. M. R., Italian, 62 years old. Pipe smoker. Leukoplasia of the upper and lower lips.

(15) Black tobacco cigarettes were smoked by (percentage figure illegible), cigars 44%, and pipe tobacco by 12% of the patients.

(16) Experimental Section

(17) In 1930, I announced the first results which I obtained from experiments on rabbits. (2) (18) At that time, I noted that neither with the nicotine extracted by us nor with pure nicotine -- its strong toxicity notwithstanding -- was I able to find epithelial lesions or tumors, despite the fact that I had conducted the experiments over a period of 8-12 months. (19) This led me to the conclusion that the carcinogenic substance of tobacco is contained in the burning residue. (20) I conducted further experiments in which I let the tobacco smoke directly affect the tissue and thus imitated the process which takes place in smokers.

(21) In a further experiment (3), we let tobacco directly affect the inner surface of the rabbits' ears. (22) Tobacco smoke is developed in a metal syringe connected to a water pump, whereby a continuous column of smoke rises from the opening of the syringe. (23) The animals were exposed to smoke 2-3 minutes per day. (24) On one of the ten rabbits, a carcinoma formed after (illegible) years, which was histologically showing a vegetative growth and was markedly cell-atypical. (25) The ear cartilage was infiltrated and penetrated. (26) Furthermore, metastases in the throat ganglia were detectable. (27) At that time, I noted that "the carcinogenic agent can be found in the combustion products" and "that has a similar effect as tar distillate." (28) I mention this here because my opinion at that time has been confirmed by the experiments described herein.

(29) In an experiment similar to the one described above, I let the tobacco smoke affect the mucous membrane of the (illegible) rabbits (4), whereby extensive leukoplastic substances were formed. (30) I attributed this effect to the resinous substances formed by the oxidation of oils and tars, which had been caused by heat generating distillation, as well as the products of resynthesis formed by (illegible) nuclei containing benzene and pyridine bases.

(31) Two years later (5), I carried out a substantial experiment on the effect of tobacco on the bladders of rats. (32) I inserted (illegible) of resin, which contained the product of tobacco combustion. (33) For control purposes, I inserted pills, containing other substances such as tar, tricresol, and aniline, in the bladders of other rats. (34) The substances obtained after a period of 8-10 months demonstrated that the pills to which the product of tobacco combustion had been added caused the most severe lesions of a purely metastastic type. (35) The histological picture clearly demonstrated that the lesions of the greatest intensity had been caused by the tar of hard coal and tobacco.

(36) A connection can be made between the results of these experiments and cancer of the bladder in humans. (37) Regarding the latter, tobacco certainly plays a role in the secretion of the combustion products. (38) Here too, it is very interesting to note that cancer of the bladder is extremely rare in women. (39) During a period of five years, 28 cases of cancer of the bladder were treated at the Institute, but among them only one was a woman, and she was (illegible).

(40) New Experiments

(41) On the basis of the experiments described above, I carried out further ones, which are discussed in the publication and which, as we shall see, not only confirm the fact that tobacco has inherent carcinogenic properties, but also that the active substances belong to the complex group which form the product known as tar from horizontal distillation.

(42) Unlike the previous experiments which were done with tobacco smoke and extracted products, these experiments were done with the substances produced by tobacco distillation applied directly to the skin. (43) Three tobacco distillation products, which resulted from the extraction at different temperatures between 0° and 400° C. were examined.

(44) First Product. (45) It is obtained by distilling tobacco in metal bowls at a temperature of 100°. (46) This temperature is maintained for the time it takes to remove the water and the light oils. (47) Then it is elevated to 120°, a temperature which is maintained for one hour. (48) The products obtained in the first distillation form a diluted yellowish liquid and of a spiritous reaction, with the characteristic odor of burning tobacco. (49) The liquid is composed of carbon dioxide, carbon monoxide, ammonia, methyl alcohol, acetic acid, acetates, pyridine, methylphyrrolidine, aldehydes, furfural, and other combustion substances which to a large part remained in the cooler.

(50) Second Product. (51) The previously obtained distillate is poured into another container, with the temperature slowly being elevated to reach 350° after two hours. (52) The distillate yielded at that temperature has the characteristic features of a conglomerate of resinous appearance, brown color and alkaline reaction. (53) The odor is strong, unpleasant and more intensive than that of the first extraction.

(54) This product, the yield of which is relatively large, since 1 kilo of black Kentucky tobacco yields 30 g., does not contain any nicotine. (55) It contains numerous substances, among which we found: carbon monoxide, carbon dioxide, ammonia carbonates, acetic acid and acetates, succinic acid, fumaric acid and fumarates, citric acids and citrates, non-characterized phenolic acids, pyrrole and its derivatives, pyridine and methylphyrrolidine, derivatives of chlorophyll, residues and resins, resynthetic substances with stable benzene nuclei (aromatic carbon hydrides, phenathrene, anthracene, benzopyrene, etc.)

(56) Third Product. (57) The third product is formed from the residue of the tobacco distillation and contains carbon and semi-carbonized residue of potassium and sodium, condensed benzene nuclei, potassium and sodium nitrates, carbonates and bicarbonates of potassium and sodium, and of smaller quantities of potassium and sodium chloride.

(58) The combustion is very restricted, given the fact that the distillation is done by direct heating and under the complete exception of air in the dead space of the apparatus. (59) Thus the distillate consists of the decomposition of the substances contained in tobacco rather than combustion products. (60) This causes a series of processes of oxidation and reduction and also of resynthesis, which account for the complex composition of the distillate.

(61) Regarding the alkaloid to tobacco, nicotine is known to boil at 245° and to decompose mainly in pyrrolidine and methylpyrrolidine, because after the first heating we found only traces of nicotine and at times not any traces. (62) The presence of nicotine can no longer be detected in the other products.

(63) The laboratory animals (rabbits) were divided into groups of 20 animals each. (64) In each of these three groups, one of the three tobacco distillation products was applied to the inner surface of the ear. (65) The rabbits in these groups were administered one dosage of the respective distillation product daily.

(66) Although after a period of ten months, none of the animals treated with the first product (a liquid substance derived from distillation at 100°to 200° C.) showed any lesions, lesions developed in a large number of the animals treated with the second product, i.e. tar obtained through horizontal distillation at 350° C. (67) Of the 20 animals in this group, 4 died during the first month of treatment. (68) 15 (94%) of the remaining animals developed tumors. (69) Just seven months after applying tobacco tar, these lesions manifested themselves in the form of small papillomas on both ears, and nine months after the beginning of the experiments, they showed the anatomical and histological characteristics of carcinomas (Figure 1).

(70) Right ear.
(71) Figure 1. (72) Tumors developed on the left ear of a rabbit treated with tobacco tar.

(73) Histological studies

(74) The histology of these lesions ranges from the simple hyperplasia of the Malpighian epithelium with considerable callous to papillomatous formations. (75) Occasionally, small papillomas or a distinctive papillomatosis with carcinogenic degenerations appear. (76) These carcinomas are characterized by infiltrative growth of the bands of epithelial tissue, strong proliferation and substantial anaplasia of the epithelial cells, which gives the tumors extreme malignancy.

(77) Figure 2. (78) Surface carcinoma with callous formations on the ear of a rabbit from Group II, which had been treated with the product of tobacco combustion. (79) The tumor was of enormous size, and its infiltrative growth reached the cartilage of the right ear.

(80) The incisions made on the same animal show the gradual progression in the evolution of the lesions. (81) After a period of only three months, metaplasia of the epithelium of the treated skin can be found. (82) Three months later purely papillomatous, verrucous growths, which show relatively little callous compared to the ones caused by coal tar, that is, 9-10 months after the beginning of the application, cancerization of the lesions has occurred.

(84) On the same ear, lesions in different evolutionary stages can be found, that is, one area of hyperplasia, various callous papillomas, and one to two carcinomas. (85) It was striking that the majority of these carcinomas had formed at the opening of the outer auditory duct. (86) I explained this by the fact that this is an area where the substance can penetrate to a greater extent, since the animals have the habit of keeping their ears in a vertical position, which facilitates the downward flow of the substance.

(87) Below I describe some of the tumors formed on rabbits treated with tobacco tar.

(88) Group II. (89) Rabbit No. 1, treated with tar (distillation). (90) The animal has seven tumors on the left ear and six on the right ear. (91) The (illegible) of the tumors has the texture of a surface carcinoma with beaded formations.

(93) Figure 3. (94) Surface carcinoma with extensive anaplasia on a rabbit treated with tobacco tar.

(95) Rabbit No. 2. The tumor on the right ear is vegetative and has the texture of a surface carcinoma with callous formations. (96) The growth is infiltrative in epithelial bands, many of which are unicellular, whereby the cells reach a state of very pronounced anaplasia. (97) One can clearly observe the individual groups of surface cells and their growth, whose type of autonomous growth is distant from the original. (98) On another ear, one sees substantial hyperplasia, marked papillomatosis and a tendency to cancerization. (Figure 3).

(99) Rabbit No. 5. (100) One of the tumors on the left ear has the texture of a surface carcinoma; its histological type is purely papillomatous-callous and consists of epithelial bands with deep, infiltrative growth.

(101) Rabbit No. 6 (102) The tumor at the opening of the outer auditory duct has the texture of a surface carcinoma.

(103) Group III. (104) Of the tumors in Group III, that is, with the final combustion product, Rabbit No. 5 shows a surface carcinoma with beaded formations on the right ear, which is characterized by extensive anaplasia of the original epithelial cells. (105) The left ear of Rabbit No. 1 of the same group shows, among other formations, a surface carcinoma with infiltrative growth on the ear cartilage, while various papillomatous formations can be found in other areas. (106) The right ear of the same animal shows a papilloma in a state of cancerous degeneration.

(107) These experiments show that the effect of the tobacco distillation products is highly carcinogenic.

(108) If, for each of the three groups, one takes into account the number of growths on one ear and the number of the animals which have developed tumors, one can see that the substances formed during the second distillation (up to 350° C.) are much more active than those formed in the third distillation which are made up exclusively of distillation residues. (109) Thus 75% of the rabbits exposed to the second distillation product developed tumors as opposed to only 70% of the rabbits exposed to the third distillation product. (110) While the early experiments have shown that the effect of tobacco smoke alone causes tumors in only 4% of the animals, this result can easily be explained by the fact that the small quantity of tobacco smoke applied to these animals contained only small amounts of the effective substances, resins and tar. (111) As mentioned earlier, these animals can only be exposed to a treatment of 3 minutes maximum daily because of the nicotine contained in tobacco smoke. (112) For this reason, the experiments using smoke alone are very lengthy: only after 2-3 years did I succeed in observing a tumor. (113) In any case, it follows that carcinogenic substances are contained in the combustion products. (114) Since rabbits can only be exposed to tobacco smoke for a few minutes because of their sensitivity to nicotine, and this causes carcinomas, would the same process not take place in humans? (115) One can easily imagine what happens to the mucous membrane of the mouth and the smoke path if they are exposed to the influence of smoke for hours.

(116) On the other hand, I am of the opinion that the carcinogenic effect of tobacco tar is related to its contents of condensed benzene nuclei and hydrocarbons of the aromatic group. (117) I will soon consider this interesting question.

(118) However, I might have already mentioned that, at my request, CORREA of the Chemistry Department of the Institute has extracted, by means of 380° C. fractionated distillation, a product from the tobacco tar which has the properties of hydrocarbons, phenanthrene, anthracene, benzopyrene, etc., and which can be characterized by a strong fluorescence of the same navy blue almost violet color, which is typical of 1-2 benzopyrene, 1-2 benzanthrene, and 1-2, 5-6 dibenzanthracene. (119) If one analyzes this tobacco product under the spectroscope, one notices an absorption in the

ultraviolet, even in high dilutions such as 0.000863% at 3870 Angstrom units, which on the other hand corresponds to the scale of absorption of the hydrocarbons mentioned, especially of benzopyrene and 1-2, 5-6 debenzanthracene. (120) The fluorescence of the hydrocarbon extracted from tobacco tar is very strong, since it is 2500 times stronger than the fluorescence used as a control substance. (121) This fluorescence has the interesting characteristic of becoming visible only under a blue filter, which is connected to the fluorescent coloring inherent in the product itself. (122) If one uses 1-2 benzanthrene as a control substance, which has a blue fluorescence as well, one can observe that under a blue filter, a very strong fluorescence is inherent in the distillate itself.

(123) All of these experiments clearly show a great similarity between the effects of coal tar and tobacco tar, not only regarding the nature of the product itself, but also regarding the cancerization in laboratory animals, and the histology and evolution of tumors in both substances.

(124) One may add that hydrocarbons are found in relatively large quantities in tobacco tar. (125) Corresponding to their physical qualities, the derivatives of phenanthrene and anthracene are predominant among the substances derived from the fractionated distillation of tobacco. (126) The presence of hydrocarbons of the aromatic group in the product of tobacco distillation cannot only be deduced from their physical qualities -- their strong fluorescence with its characteristic coloring and its resinous composition -- but also from the nitrate and sulfonate derivatives, which develop through the reaction of the respective acids inherent in cyclic nuclei.

(127) Finally, I would also like to mention in this regard that in Dr. A. E. Roffo, Jr.'s spectrograms of various hydrocarbons of carbon and tobacco distillates, the absorption of the tobacco derivative is complete, despite the fact that the band of dibenzanthracene is missing in the tobacco distillate.

(1) What one should know about cancer. Information pamphlet. Buenos Aires. 1928. (2) Z. Cancer Research 1931, Vol. 33. (3) Bull. Inst. Med. Exper. Buenos Aires, 1932, No. 28. (4) Bull. Inst. Med. Exp. Buenos Aires, 1930, No. 23. (5) Bull. Inst. Med. Exp. Buenos Aires, 1932, No. 27.

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