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Roffo AH: Carcinoma desarrollado en un conejo fumador a lost res anos.  
Bol Inst Med Exp 7: 545-573, 1932. [SOA 225; TDH Exhibit 58, 59; Cited  
P81, P175, P229, P235]

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(11) Carcinoma Developed in a Smoking Rabbit  
(12) at three years  
(13) by Dr. A. H. ROFFO

(14) The numerous and worthy clinical observations of carcinoma localizations in the mouth, larynx and lungs produced in heavy smokers, give tobacco the value of a carcinogenic agent.

(15) It is in the sense of studying the effect of its action on epithelial tissue that I have oriented the experiments; because if the relationship is one of cause and effect, there appear to be some sufficiently pure clinical cases that the experiment would not have produced in a categorical form.

(16) The mortality rate yielded by the attendance of patients at the Institute's facility, signals that the localization of carcinoma in the mouth (lips, tongue, cheeks, etc.) and larynx is of a frequency that is highly variable according to sex. (17) Very frequently in men, so much so that it predominates in the absolute; while in women, its observation is a rarity, in such a way that out of 8,000 female cancer patients I have observed, I have only found 4 of this last localization, with the addition that these 4 were heavy smokers.

(18) It is not possible to attribute to a cause other than cancer the differences that are presented with regard to this localization as opposed to another, as for example that of the stomach, in which the percentage is high for both sexes, as is shown in the following comparative figures:

(19) <u>Localization</u>	<u>Men</u>	<u>Women</u>
(20) Mouth	92.4%	7.5%
(21) Stomach	57.6%	42.4%

(22) Well now, if we rely on the figures observed for men only, for the mortality of patients treated at the Institute, cancer of the mouth and larynx is not only that which yields the highest percentage, but is also that which presents tobacco use in its meticulously taken history in such a way that 92% were heavy smokers, a figure which gives tobacco an undeniable value as a carcinogenic agent.

(23) Carcinomas of the mouth and larynx:

(24) Heavy smokers	92%
(25) Moderate smokers	8%

(26) Undoubtedly, and in the face of this information, it comes forth as a question, why not all smokers necessarily develop cancer.

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(27) In accordance with the experiment produced in the last few years, above all with biochemical studies, one deduces that the concurrence of two factors is necessary to the cancerization of a tissue: one which refers to the biological foundation, and the other which is related to incidental cause, that acts locally to unleash the atypical, anarchical cell growth. (28) The first condition is a product of biological modifications of the tumors that, biochemically altered, produce energetic substances that stimulate cellular growth; while the second translates into an irritating agent that can be physical, mechanical, parasitical, or chemical and among which one finds tobacco.

(29) The concurrence of these two factors is indispensable so that the cancerization of irritated tissue is produced, and this gives us as well the reason why there are species of animals more sensitive to the production of experimental cancer, as it happens with tar it develops easily in some species (rabbits and mice), and not in others, even if they are similar (guinea pigs and rats).

## II

(30) In the experimentation that I have developed with tobacco, I have already indicated the production of leukoplasic lesions in rabbits, by causing the action of tobacco combustion products (smoke), in chronic form and the results of which I made known in 1930. (1).

(31) In another experiment developed using the action of combustion products found in smoke, from which various products have been extracted, I have obtained the development of carcinoma in a rabbit's ear, after nine months of application of the aqueous solution obtained (2), resulting from an eptithelial neoplasia, of vegetative and infiltrative growth, with metaplasia and discontinuous growth.

## III

(32) In accordance with the experimentation previously developed, I attribute the carcinogenic action of tobacco to the combustion products that act in the same manner as the distillation products of tar, amongst which are found: essential oils, pyridine and its derivatives, empyreumatic substances as well as mineral compounds.

(1) A. H. Roffo, Experimental Tobacco-induced Leukoplasia, Bulletin of the Institute for Experimental Medicine, Volume VII, Number 23, page 130, 1930.

South American Review of Medicine and Surgery, Volume I, Number 4, page 321, April 1930.

(2) A. H. Roffo, Development of a Carcinoma in a Rabbit by Tobacco, Bulletin of the Institute for Experimental Medicine, Volume VII, Number 24, page 502, 1930.

Zeitsch. f. Krebsforschung, Volume 33, Number 4, page 321, 1930.

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(33) And I must hold fast to this, even more to this concept, taking into account the negative results I have obtained working with nicotine, already in extract form all extracted cold from tobacco leaves and from pure nicotine, without combustion; with which only irritative lesions have been obtained, with some hyperplasia, but always with serious general intoxication.

(34) In light of this and to bring ourselves as closely as possible to what happens in the practice of smoking, I have undertaken this experimentation making the smoke from burning tobacco act directly upon the inner surface of a rabbit's ear.

(35) To do this, one places in a metal syringe, burning tobacco, the smoke of which exists in continuous form through the influence of a water-filled tube.

(36) The general state of the animals treated in this manner suffers considerably, with rapid weight loss. (37) If the insufflation is done daily, they die within 5 to 6 days, for which it is convenient to space out the applications during the first days. (38) These must be short, a total of two minutes; if they are more prolonged, the animal collapses and dies.

(39) Out of the 10 animals thus treated, one died after three years of treatment. (40) In the autopsy conducted by Assistant Garcia Velloso, one finds in the site of tobacco smoke influence, an ulcerous lesion with a red, vegetative bottom, and around this in its upper portion a nodule the size of a pea, another similar one in the lower portion and disseminated in this central zone of the ear, irregular hyperkeratous scabs. (41) (See Illustration 1).

(42) These formations present, in the histological examination, the undeniable characteristics of a carcinoma, not only for the intensity of the epithelial growth, but also for the condition in which this takes place of an autonomous and anaplastic character and whose development reaches and penetrates the central ear cartilage.

(43) In the carotid region, there is a hard ganglion the size of a pea, which when one cuts, one sees that it is formed, in large part, by a tissue of a consistent whitish color.

(44) Histological description -- Examining the nodule that is found in the upper portion of the ear, one observes a purely papillomatous zone (Microphotograph 2) and in which one can appreciate a process of growth that goes from the epithelial hyperplasia to the papillomatous, hyperkeratous formation, with a deep, epithelial growth, see details (Microphotograph 3 to 11).

(45) The histology of the central, ulcerous portion (Microphotograph 12) corresponds to two types of lesions: one which is found at the edges and presents a vegetative, a papillomatous aspect (Microphotograph 14) with abundant formations of hyperplastic epithelium that enlarges the Malpighian body in an accentuated form, from which are issued epithelial bands that form an intricate network of deep growth.

(46) The alterations that comprise this central portion of the preparation are characterized by an epithelial growth, purely infiltrative, that reaches the cartilage in its proliferation. (47) It is interesting to observe the characteristics that this epithelial growth acquires as it goes from the simple hyperplastic

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formations (Microphotograph 18), in which one can observe the existence of numerous isolated epithelial cells and with autonomous growth, through the formation of keratous beads, many of which are encountered at a depth (Microphotograph 19), and that in the preparations of this area cut in a series, one can appreciate that this hyperkeratous, epithelial formation reaches in this profound growth to the cartilage and penetrates it, which can be seen in (Microphotograph 20), observed at greater magnification, this same epithelial formation with its concentric keratous mantles and around this some isolated epithelial cells in the connective mass.

(48) (In these Microphotographs 21, 22, 23, 24, 25, 26 and 27), the area is represented that corresponds to the other preparation and in which some anaplastic, epithelial groups are observed in the area of the cartilage or infiltrating the space of the same.

(49) In Microphotograph 28, one can observe three groups of neoplastic cells resting upon the cartilaginous band, the tendency of these cells to keratosis appearing in one of these (e).

(50) ILLUSTRATION I - Rabbit's ear with multiple lesions: a, healthy region (see Microphotograph 1); b and d, papillomatous formations; c, ulcero-vegetative lesion.

(51) ILLUSTRATION II - Section of the ear, with multiple lesions that go from the hyperplastic epithelium: a, deep growth; b, discontinuous growth; c, invading growth with bead formation; d, and hyperkeratosis e.

(52) ILLUSTRATION III - Anaplastic epithelial growth, formation of beads and isolated epithelial group between the cartilage (a).

(53) In the carotid ganglion to which I have previously made reference, one observes (Microphotograph 29) that the central zone is occupied by neoplastic tissue, that completely infiltrates it with invasion and destruction of the adenoid tissue. (54) This, observed at greater magnification, corresponds to neoplastic elements (Microphotograph 30) of the epithelial type, very deviated and purely anaplastic.

#### IV

(55) The lesions previously described are themselves from an evidently neoplastic proliferative process, and they correspond to the distinct phases that characterize a neoplastic epithelium: hyperplasia, vegetative and infiltrative growth with metatypia, discontinuous and autonomous growth.

(56) The character of the carcinoma that I assign to this process derives, not only from the conditions previously indicated, but also from others that have greater importance and leave no room for doubt. (57) I refer to the form of anarchical growth, that in the central lesion is characterized by:

(58) a) unicellular rows and unattached cells to which, therefore, we must ascribe an autonomous growth;

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(59) b) a metatypia that the epithelium acquires in its growth, taking a very anaplastic form;

(60) c) the intensity of this growth that reaches and penetrates the cartilage.

(61) d) and, finally, the formation of ganglionic metastasis on the side of the primary lesion.

(62) Well now, we are dealing with a tumor whose development is strictly related to the irritative agent employed, the carcinogenic value of which has been clinically validated and that in this case becomes evident at the site of application, after three years of treatment.

(63) As in the case of the rabbit to which I made reference above, I consider that the carcinogenic agent of tobacco is found in the combustion products, acting thus in a manner similar to the distillation products of tar. (64) I refer especially to the quantity of substances that are contained in tobacco smoke, such as essential oils, pyridines, empyreumatic bodies, carbon monoxide, etc., having very intense pharmacodynamical effects.

(65) Microphotograph 1 - Section of the rabbit's ear from the upper zone, appearing with its normal structure.

(66) Microphotograph 2 - Area with hyperplastic epithelium and papillomatosis.

(67) Microphotograph 3 - Continues of the previous one, with hyperplastic epithelium in a) and in b) hyperkeratous, papillomatous epithelium.

(68) Microphotograph 4 - Of the previous preparation, at greater magnification; hyperkeratous, hyperplastic epithelium.

(69) Microphotograph 5 - Of Microphotograph 3, at greater magnification; papillomatosis with keratous formations.

(70) Microphotograph 6 - Of Microphotograph 3, hyperplasia and infiltrative growth.

(71) Microphotograph 7 - Of Microphotograph 3, hyperplasia and papilloma.

(72) Microphotograph 8 - Section of a papilloma on the inner surface of the ear where hyperplasia and a profound, initial growth can be seen.

(73) Microphotograph 8 - Of the previous preparation, hyperplasia with keratosis and deep growth.

(74) Microphotograph 9 - Of the preparation of the previous papilloma, hyperplasia, hyperkeratous papillomatosis and deep growth.

(75) Microphotograph 10 - Of the previous preparation, at greater magnification, hyperplasia and keratosis.

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(76) Microphotograph 11 - Of the previous preparation, vegetative, papillomatous formation with keratosis.

(77) Microphotograph 12 - Preparation of the central ulcerous portion. (78) Purely carcinogenic structure. (79) Vegetative, profound epithelial growth with keratous spheres. (80) In a) one observes an epithelial outgrowth with anaplastic elements and located in the space of the cartilage.

(81) Microphotograph 13 - Corresponds to portion a) of the preceding preparation.

(82) Microphotograph 14 - Of preparation 12, papillomas with hyperkeratosis and profound, epithelial growth.

(83) Microphotograph 15 - Of preparation 12, keratous papilloma and profound growth.

(84) Microphotograph 16 - Another preparation of the central, ulcerous zone in which one observes the purely cancerous aspect of the lesion, with epithelial outgrowths near the cartilage.

(85) Microphotograph 17 - One of the outgrowths to which reference was made in the preceding microphotograph, observed at greater magnification.

(86) Microphotograph 18 - Profound growth of the epithelium, in which unicellular rows occur, one can see numerous cells of this type, isolated in the connective stroma.

(87) Microphotograph 19 - Inner portion of the preparation, in which one observes that the epithelial growth is discontinuous with the formation of numerous keratous beads.

(88) Microphotograph 20 - Of preparation 12, in which one observes the epithelial growth in anarchic form: isolated cells in unicellular rows and in groups.

(89) Microphotograph 21 - Another aspect of the preceding preparation, where one sees the anaplastic epithelial growth.

(90) Microphotograph 22 - Of preceding preparation, in which the anaplastic aspect of the growth is more marked, the epithelial cells that appear isolated being numerous.

(91) Microphotograph 23 - Of the same central zone where one observes an epithelial bead in the ear cartilage a).

(92) Microphotograph 24 - Area a) of the previous preparation at greater magnification. (93) Keratous epithelial bead in the middle of the cartilage.

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(94) Microphotograph 25 - a) anaplastic, epithelial group in the space of the cartilage.

(95) Microphotograph 26 - Area a) of the previous preparation, seen at greater magnification.

(96) Microphotograph 27 - Groups of very anaplastic epithelial cells near the cartilage.

(97) Microphotograph 28 - Groups of epithelial cells (a-b-c) resting upon the ear cartilage.

(98) Microphotograph 29 - Total section of the ganglionic carotid, that shows a neoplastic metastasis in the central zone.

(99) Microphotograph 30 - Of the central zone of the preceding preparation. (100) Neoplastic epithelium whose cell type appears to be very anaplastic.

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*Louise B. Smith, Director*

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