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1:2 - Benzopyrene

Carcinogenic Extraction of Tobacco Tar

By Dr. Angel Roffo

(1) In earlier reports, I have discussed the carcinogenic action of tars extracted from different tobaccos available commercially; of black tobaccos such as Havana, Kentucky, Paraguayan, Saltan and twist; and of blond tobaccos, such as Egyptian and Turkish.

(2) These tars occur as a product with similar physical and organoleptic characteristics. (3) The product is a thick, resinous conglomerate, with a blackish coloring and which discharges an intense, unpleasant odor characteristic of burning tobacco. (4) These characteristics are even more pronounced in blond tobacco tars.

(5) Different kinds of tobacco produce between 41 and 110 grams of tar per kilo of dry leaves.

(6) The carcinogenic action of these tobacco tars has been related to the existence of hydrocarbons of the aromatic group in this product.

(7) The complex obtained through distillation contains highly fluorescent bodies whose navy blue, almost violet color is characteristic of other carcinogenic hydrocarbons.

(8) The tars are further characterized on the U.V. spectrograph by numerous absorption bands on the scale of carcinogenic hydrocarbons.

(9) However, these tars represent a complex product with numerous components, necessitating a strong dilution in order to avoid the obstacles of the superposition of the bands of different components.

Carcinogenic Action of Hydrocarbon Tobacco Tar

(10) In eight of the previous results, the current experiment was developed in order to determine which substance in tar has a carcinogenic function. (11) To do so, a hydrocarbon with the physical and chemical characteristics of benzopyrene was extracted, using a split distillation process.

(12) In the article referred to above, I have already stated that "the carcinogenic action of tobacco tar is related to the presence of condensed benzene nuclei of hydrocarbon bodies in tar of the aromatic group." (13) "The bodies in tar are characterized not only by their strong fluorescence, but also for the fluorescent color which is characteristic of other carcinogenc hydrocarbons, such as (2) benzopyrene, 1-2-5-6 dibenzanthryacene, etc. (14) Another characteristic which establishes a close relation with the hydrocarbons mentioned is that a spectrographic examination is that a

(1) Unidad carcerigena de los Alcatranes de diversos tipos de tobacos. Bol. del Inst. de Med. Exp. No. 48, pag. 349, 1938.

a spectrographic examination reveals a U.V. absorption of numerous bands which are produced between 2,000 and 4,000 Angstrom U. (A). which coincides with the scale of absorption of the above-mentioned carcinogenic hydrocarbons.

(15) The results of this experiment, to be discussed in this report, confirm the concept which I proposed at that time; through the experiment, a distilled product with the characteristics of florescence and spectrography of carcinogenic benzopyrene was obtained and has produced tumors in rabbits' ears at a rate as high as 100%.

(16) The relationship of this substance obtained from tobacco is the following:

The presence of pyrene (C16H10) and its tar derivatives has been determined both physically and chemically.

(17) Its formula indicates that it is a polycyclic hydrocarbon with 4 condensed benzene nuclei, positioned in such a way that they could be considered a naphathalene derivative, to which 2 groups: + CH - CH = CH - have been incorporated in the 4 carbons in position for (1:8 and 4:5).

[GRAPH]

Pyrene Benzopyrene

(18) The substance in question has acquired importance ever since the Kentucky tobacco demonstrated, in 1924 that the tar distillation products at 500° C. (760 mm) possess carcinogenic activity.

(19) Then, the characterization through spectrographic analysis of the pyrene and its derivatives presented a series of difficulties which have since been overcome. (20) Thus, Mayneord and Roe (2), using a high dispension spectrograph, do not find visible differences between the spectrum of fluorescence in samples of 2:4 benzopyrene of variable purity.

(21) It was necessary to use the Sannie microphotometer (3) in order to see the differences in the spectrographs of 3:4 benzopyrene which previous authors had not been able to determine.

(22) However, this author concludes that, when the product is found in complex mixtures, as in the case of tars, the microphotometer does not solve the problem.

(23) Miescht (4) also uses the microphotometer, but concludes that the substances mentioned above can be attributed to the fact that very concentrated solutions were used. (24) The results obtained using diluted solutions demonstrate that the absorption bands of 3:4 benzopyrene were not affected by the presence of a quantity five times greater of 1:2, 5:6 dibenzanthracene.

(2) Biochem. I. 24, 497 (1930).
(3) Biochem. I. 30 (1936).
(4) Biochem. Ztscr 287, 189 (1936).

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(25) But in order to characterize the presence of 3:4 benzopyrene in tar distillation products, Hieger (4) based on Mayneord's observation, refers to the florescent spectrum, relative to the intensity of the two bands located on 4040 and 4270 Angstrom units of 3:4 benzopyrene and which predominate over the bands other combined substances.

(26) The substance used in this experiment was obtained above 380 C. by a split distillation process of tobacco tar which presents highly carcinogenic properties. (27) It was prepared using the following procedure by Dr. L. M. Correa in the Chemistry Department of this Institute:

(28) The procedure for obtaining tobacco tar which has been followed at the Institute for the past 3 years is as follows: through a process of pyrogenic distillation of dry leaves in a non-oxidizable steel container at normal pressure (5). (29) The product is kept in a stove at 120 C. for 6 days in order to eliminate the more volatile substances. (30) At the same time, the residue acquires through oxidation the aspect and smell of tar which indicates its origin.

(31) The tobacco tar obtained in this way is distilled in a 760 mm glass flask by slowly raising the temperature, which requires constant attention. (32) When the boiling point is reached, the temperature is raised; first the products which distill and dissolve at a temperature below 380 C. are separated in order to gather those products being distilled above 380 C. and to the point of residue carbonization.

(33) The oil obtained above at this temperature is distilled two more times by the same procedure described above.

(34) During this distillation, two portions are once again obtained: one which distills below 380 C., and one above 380 C.

(35) It is this last portion, distilled above 380 C., which has been used in the experiments for this report; its fluorescent and spectrographic characteristics, which will be presented below, show the presence of 1:3 benzopyrene.

Spectrographic Study:

(36) The spectrographic study of this substance, undertaken by Dr. A. E. Roffo (Jr.), indicates the following characteristics according to the different dillutions used to obtain the results:

(4) The Al. C.I. of Cander. 29, 715 (1937).

(5) ROFFO, A.H. El Tobaco Como Cancerigena. Bol. del Inst. de Med. Exp. No. 42, pag. 287, 1936. -- El Tobaco Rubio come Cancerigeno. Bol. del Inst. de Med. Exp. No. 47, pag. 5, 1938.

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(37) (See table, p. 5 of original text)

(38) Observations:

There are several absorption bands included in the following regions.

No. 1 between 2490 A.U. and 2555 A.U.
No. 2 between 2580 A.U. and 2660 A.U.

(39) (See table, p. 5 of original)

(40) Observations:

There are several absorption bands included in the following regions.

No. 3 between 2477 A.U. and 2565 A.U.
No. 4 between 2565 A.U. and 2642 A.U.
No. 5 between 3055 A.U. and 3124 A.U.

(41) (See table on p. 6 of original).

(42) Observations:

There are several absorption bands included on the following regions.

No. 6 between 2488 A.U. and 2555 A.U.
No. 7 between 2615 A.U. and 2646 A.U.

(43) (See table on p. 6 of original.)

(44) Observations:

There are several absorption bands included in the following regions:

No. 8 between 2084 A.U. and 2138 A.U.
No. 9 " 2160 A.U. and 2204 A.U.
No. 10 " 2396 A.U. and 2420 A.U.
No. 11 " 2620 A.U. and 2658 A.U.
No. 12 " 2700 A.U. and 2762 A.U.
No. 13 " 2762 A.U. and 2935 A.U.

(45) Spectrograph No. 1
U.V. spectrograph of the product extracted from tobacco.

(46) Spectrograph No. 2
U.V. spectrograph of the product extracted from tobacco.

(47) Spectrograph No. 3
UV spectrograph of the product tar at 1%.

(48) Spectrograph No. 4
UV spectrograph of the product tar at 1%.

(49) For a better interpretation, we have summarized the previous results in the following way:
(50) It can be observed that the absorption bands are produced according to dillution within the following units:
(See table p. 9. of original)

(51) From the above, it is clear that bands: 1-3 and 6, which correspond to dillutions of 1 /o, 1/oo, and 1 5/oo, are similar; the same is true for bands 2 and 4, and bands 7 and 11.

(52) Band 8 corresponds to a similar one of anthracene.
(53) Band 9 corresponds to a similar one of 1:2 benzopyrene.
(54) The same can be observed with band 10, 1-3 and 6, and 2-47 and 11, 12 and 5.
(55) Band 13 corresponds to a similar one of phenanthrene.
(56) Therefore, this product is made up primarily of 1:2 benzopyrene and secondarily of phenanthrene and anthracene.

(GRAPH)
(See figure p. 10 of original).

(57) Ultraviolet absorption of chemically pure 1:2 benzopyrene (1) and benezopyrene extracted from tobacco tar (2).

FLUORESCENCE

(58) Moreover, the fluorescence of this product shows an intensity and coloring similar to that of benzopyrene, as can be seen in the following summary of the results obtained.

(59) Standard of Fluorescence: Fluorescent cyrstal = 100

Filters	Tobacco derivative in ether solution				Benzopyrene 10/000 ether	Phenanthrene 1 0/000 ether
without filter	98	120	235	360	320	263.2
L1	0	0	0	0	0	0
L2	0	0	0	0	0	0
L3	122	248	450	700	625	3333

THE CURRENT EXPERIMENT

(60) This experiment was performed using rabbits, and the same technique used previously to obtain carcinoma from pure tobacco tar.

(61) That is to say, the skin of the inner surface of the right ear was treated every other day.

Results of the Current Experiment

(62) The tumorous lesions have developed in all of the animals, or 100%.
(63) The initiation of the process varies; in some animals, papillomatous lesions were observed after two months and 15 days.
(64) In general, however, the first manifestations occur between 5 and 7 months.
(65) This experiment was developed with a sampler of 18 rabbits. (66) As can be seen in the summary below, the first lesions appeared anywhere between 2 and 7 months.

(See chart p. 11 of original).

Rabbit Number	Time of appearance of the first lesion

(67) In order to determine the influence of cholesterol on the area, and therefore on the precocity and development of the lesions, we have submitted a group of six animals to a cholesterol treatment - 1 subcutaneous injection of 10% oil solution every other day.
(68) It was observed that in these rabbits, the lesions not only appear far earlier than in the others, but also prove more malignant in growth, as can be seen in the following summarized chart:

(CHART See p. 12 of original).
(69) Appearance of the first tumorous lesions according to the cholesterolization of the area.

1st papillomatous lesion	Animals with cholesterol injection	Animals Without cholesterol injection

(70) By examining the percentages above, it is clear that the number of animals afflicted with cancer by this hydrocarbon distilled from tobacco tar represents the highest number of those obtained so far. (71) If with pure tobacco tar, the rate of cancer varied from 80-90%, with this product, it became 100%.
(72) Now then, as for the appearance of the lesions, this points out a larger pattern, thus establishing a notable difference between animals with a cholesterol injected area and those with a normal area.
(73) The results show that, in the first group, the papillomatous precancerous lesions which appeared within 2 months and 15 days constitute 50% of the group, whereas in the second, non-cholesterol injected group, the lesions only constitute 9.99%.

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(74) It can also be observed that all of the animals in the cholesterol injected group show lesions at 6 months, whereas in the other group, the percentage is 9.09%, at a time when they had recently become cancerous.

(Figure)
Chart summarizing the developed tumors: Group 1: rabbits' ears not injected with cholesterol. -- Group 2: Rabbits' ears injected with cholesterol.

Histopathogy of the lesions produced.

(76) In the histological study of the lesions developed, a histopathogical type similar to the tumors produced with pure tobacco tar can be observed. (77) These are neoplastic productions which infiltrate and destroy neighboring tissues and even the ear cartilage some which are vegetative, others which are infiltrative and grow to an enormous size in relation to the organ in which it has developed.
(78) On the other hand, these are tumors which derive from the Malpighian epithelium of the skin and are of the spino-cellular type.
(79) The initiation of the process has presented the same characteristics in all of the animals, and can be superimposed on the description carried out in the evolution of the lesions obtained with tobacco tar.

This evolution can be divided into 3 periods:

(80) 1st) Hyperplasia of the Malpighian epithelium in the zone where the hydrocarbon was injected; the process is characterized by an enlargement of the dermis and a marked cornification of the more external layers. (81) Histologically, this period is manifested by a hyperplasia of the spino-cellular epithelium, which is 4-5 times thicker than the adjacent cutaneous epithelium and has a marked hyperkeratous production.
(82) 2nd) The period described above, which lasts 1-2 months, is followed by another characterized by the production of papilloma, which occur in an isolated fashion of 1 or 2, and, at times, as a papillomatosis of numerous units.
(83) In the histological study of this period, it can be observed first of all that the hyperkeratosis is highly pronounced. (84) Epithelial rashes are formed simultaneously, many of them dichotomized, vegetative and with keratous surface epithelial layers.
(85) In the histological preparations, a microscopic papillomatous formation often occurs in the form of small philamentous rashes, which are, no doubt, precursors of other future tumors.
(86) This period is characterized by the cancerization of these formations, a process which takes place within a year to a year and a half in the growth of the lesions. (87) In this stage, the characteristics of malignant neoplasm become clearly defined: intense proliferation, infiltration and destruction of the organ, and the subsequent appearance of neoplasm in the opposite side of the ear.

(88) Histologically, an external hyperkeratous epithelial growth is usually observed at the beginning; this later develops into an internal growth in epithelial rashes which infiltrate the dermis with discontinuous growth. (89) In many of these tumors, one can observe a marked anaplasia from the original epithelium, a factor which gives these tumors such a highly malignant quality.
(90) The animals die within 18-24 months after the beginning of the experiment.

(91) In the following, I will describe some of the tumors which developed, placing them within the different varieties of evolution of the process
(92) Rabbit No. 2 - At 2 months, 17 days, this animal developed a pea-sized papilloma at the tip of the right ear; a month later it grew to be the size of a nut. Photograph No. 1.
(93) Another tumor developed in the central portion of the ear and shows signs of being a vegetative papilloma.
(94) This animal died in the 2nd year, at which point the tumor had reached an enormous size in comparison with the organ; it could be compared in volume to a mandarin orange. Photograph No. 2.
(95) It is made up of a soft, compact tissue, with areas of hemorrage; it has grown to include the ear cartilage.
(96) The histology of this neoplasm presents a very interesting characteristic, that the formation of new tissue includes the conjunctive and the epithelial tissues, both of which are fundamental.
(97) The enormous tumorous mass is primarily formed from a sarcomatous tissue, of the fusocellular kind (Microphotograph No. 10.) within this formation are found zones formed by surface epithelial rashes, with many keratous spheres. (Microphotograph No. 2). (98) It would seem to be that a mixed neoplasm of the carcino-sarcomatous kind of which I described others similar to these in a study of histopathogeny of carcinoma produced by the sun. (99) This observation shows that both of these species of cells can react equally to the carcinogenic hydrocarbon.
(100) Another tumor formed by surface epithelium has developed in the ear of this same animal. (101) The passage from hyperkeratosis to cancerization of the cutaneous Malpighian epithelium can be observed in Microphotograph No. 3; the cancerous style is represented by the central nodule.
(102) The discontinuous epithelial growth, both in bands and in isolation can be observed in microphotographs Nos. 4 and 5. (103) Here, in addition to earlier characteristics, the marked anaplasia of the Malpighian epithelium is present.
(104) Rabbit No. 17 - This animal has developed a highly malignant carcinoma; the papillomatous formations of the first period have been succeeded by an intense infiltration which invaded the entire organ, massively destroying tissues throughout almost half of the organ. Photograph No. 3. (105) This animal died at one year and six months.

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(106) The histology of this tumor corresponds to the surface type of carcinoma with marked epithelial anaplasm as well as -- discontinuous growth, which can be observed in Microphotographs No. 6, 7 and 8.

(107) Rabbit No. 16 - This animal has developed 2 tumors in the central portion of the ear; they are papillomatous, vegetative and infiltrative. (Photograph No. 4). (108) This rabbit died at 7 months.

(109) The histology of this neoplasm is the same as that described above. (110) In Microphotograph No. 9, one of the nodules with different stages of transition in the cutaneous Malpighian epithelium can be observed. (111) In Microphotograph No. 10, the other cancerous nodule showing the same histological type, with infiltrative growth and a marked anaplasia of the epithelium can be observed. (Microphotograph No. 11).

(112) Rabbit No. 13 - This animal has developed a vegetative ulcerous tumor in the upper tip of the ear (Photograph No. 5) (113). This animal died at 7 months.

(114) The histology reproduces the structure of the previous tumor (Microphotograph No. 12) (115) It is a surface carcinoma with keratous spheres, infiltrative growth and marked anaplasia. (Microphotograph No. 13).

(116) Rabbit No. 10 - This animal developed three tumors in the lower portion of the year (Photograph No. 6). (117) It died at 7 months.

(118) The histology reproduces the structure of the above tumor (Microphotograph No. 14). (119) It is a surface carcinoma, with keratous spheres infiltrative growth and a marked anaplasia. (Microphotograph No. 15).

(120) Rabbit No. 1 - This animal developed a tumor the size of a seed in the central portion of the ear, as well as 3 papillomas distributed along the edge of the ear, and on the inner surface (Photograph No. 7). (121) This animal died at 2 years, 7 months.

(122) The histology reproduces the above tumor (Microphotograph No. 16). (123) It is a surface carcinoma with keratous spheres, infiltrative growth and a marked anaplasia (Microphotographs 17 and 18).

(124) Rabbit No. 14 - This animal developed a vegetative, ulcerous tumor in the central portion of the car, and another the size of a chick-pea in the lower portion. (125) There are also 5 papillomas distributed along the surface of the ear. (Photograph No. 8) (126) This one died at 7 months.

(127) The histology reproduces the structure of the above tumor (Microphotograph No. 12). (128) It is a surface carcinoma with keratous spheres infiltrative growth and a marked anaplasia (Microphotograph No. 20).

(129) Rabbit No. 5 - This animal developed a vegetative, ulcerous tumor in the lower part of the ear (Photograph No. 9). (130) The animal died at 7 months.

(131) The histology reproduces the above tumor (Microphotograph No. 21. (132) It is a surface carcinoma with keratous spheres, infiltrative growth and a marked anaplasia. (Microphotograph No. 22)

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(Photographs)

Photograph No. 1
Tumor the size of a nut, at four months.

Photograph No. 2
Enormous tumor the size of a mandarin orange, with invasion of the external surface of the ear.

Microphotograph No. 4
Magnification of the preceding photograph in which one observes the discontinuous growth and a marked anaplasia.

Microphotograph No. 5
Preceding photograph at a greater magnification in which one observes the discontinuous growth and a marked anaplasia.

Rabbit No. 17
Photograph No. 3 - Enormous tumor developed at one year and six months, with invasion and deterioration of the organ.

Microphotograph No. 6
The edge of the preceding ulceration; surface carcinoma with markedly anaplasial epithelia.

Microphotograph No. 7
Discontinuous, infiltrative growth near the ear cartilage.

Microphotograph No. 8
Markedly carcinomatous infiltration which characterized by isolated elements (missing).

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Rabbit No. 16
Photograph No. 4 - 2 vegetative and infiltrative tumors, developed at 7 months.

Microphotograph No. 9
Carcinomatous nodule of a small tumor.

Microphotograph No. 10
Carcinomatous nodule of a larger tumor.

Microphotograph No. 11
Preceding photograph at greater magnification, one observes the discontinuous, infiltrative growth of the epithelium.

Rabbit No. 13
Photograph No. 5 - Vegetative tumors, developed at 7 months.

Microphotograph No. 12
Histology of the preceding tumor: surface carcinoma.

Rabbit No. 10
Photograph No. 6 - 3 tumors developed at 7 months. One of these small tumors is of the surface, carcinomatous type.

Microphotograph No. 14
(missing) preparation of a small tumor: surface carcinoma.

Microphotograph No. 15
The preceding preparation at greater magnification, in which one observes the discontinuous and anaplasial growth.

Rabbit No. 1
Photograph No. 1 - 1 tumors the size of a strawberry, furthermore, one observes 8 papillomas distributed across the rest of the ear.

Microphotograph No. 17
The preceding photograph at greater magnification, in which one observes the infiltrative and anaplasial growth.

Microphotograph No. 18
No caption.

Rabbit No. 14
Photograph No. 8 - T: vegetative, ulcerous tumors, also 5 papillomas exist.

Microphotograph No. 19
Tumor T: surface carcinoma.

Microphotograph No. 20
The preceding preparation at greater magnification, in which one observes the discontinuous and anaplasial growth.

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Rabbit No. 5
Photograph No. 9 - T: vegetative, ulcerous tumors, developed at 7 months.

Microphotograph No. 21
Preparation of the previous tumor, surface carcinoma with rounded growths.

Microphotograph No. 22
The previous preparation at greater magnification, in which one observes the discontinuous and anaplasial growth.

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