

EXPERIMENTAL CARCINOMA OF ESOPHAGUS
I-EFFECT OF NASS IN THE SQUAMOUS EPITHELIUM OF
ESOPHAGUS IN RATS AND MICE.

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Introduction

The inhabitants of Turkamansahra, in the North Eastern part of Iran chew a substance which is called NASS. The epidemiological studies*** of cancer of the digestive tract specially that of esofagus in Iran shows that a great number of the sufferers belong to this part of Iran. The incidence of this disease in men and women both young and old is high.

The reports of Wahi (40) in 1963 shows that this substance is consumed in Central Asia too, and he has also mentioned cancers of the oropharynx which are induced by the above mentioned substance.

Historical Review.

There are more than 25 reports which prove the relation between tobacco and bronchial carcinoma.

The conclusion is arrived after study of several thousand cases of persons suffering from this type of Cancer. In addition there are a lot of documents which confirm that there is a connection between tobacco and cancers of the lip, mouth, throat, pharynx, stomach and finally the bladder. (18. 20. 23. 27. 34. 39.).

The first experiment about the carcinogenic effect of tobacco was done by Roffo (1930) in which he rubbed tobacco on the skin of rabbits for a long period of time and got a leukoplasic condition. In Roffo's opinion these epithelial changes were a precancerous state.

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Roffo in 1932 by putting the smoke of cigarettes in the ear of rabbits, two minutes per day for a period of three years, induced the first cancer accompanied by metastasis in the lymph nodes. In 1936 he proved that tobacco tar produces some type of papillomas in the ears of rabbits which changes into a cancerous lesion within a 10 months period. The results of his experiment showed that yellow tobacco (e. g. Turkish & Egyptian) was more carcinogenic, because of its toxicity. In 1939 Roffo proved that the carcinogenic effect of tobacco is obvious but sometimes certain types of tobaccos (e. g. Oriental tobaccos which are consumed by chewing) have more carcinogenic effects. (31. 32.).

In 1931 Chikomatsu after rubbing tobacco tar on the ear of the rabbits for a long period of time got carcinoid lesions.

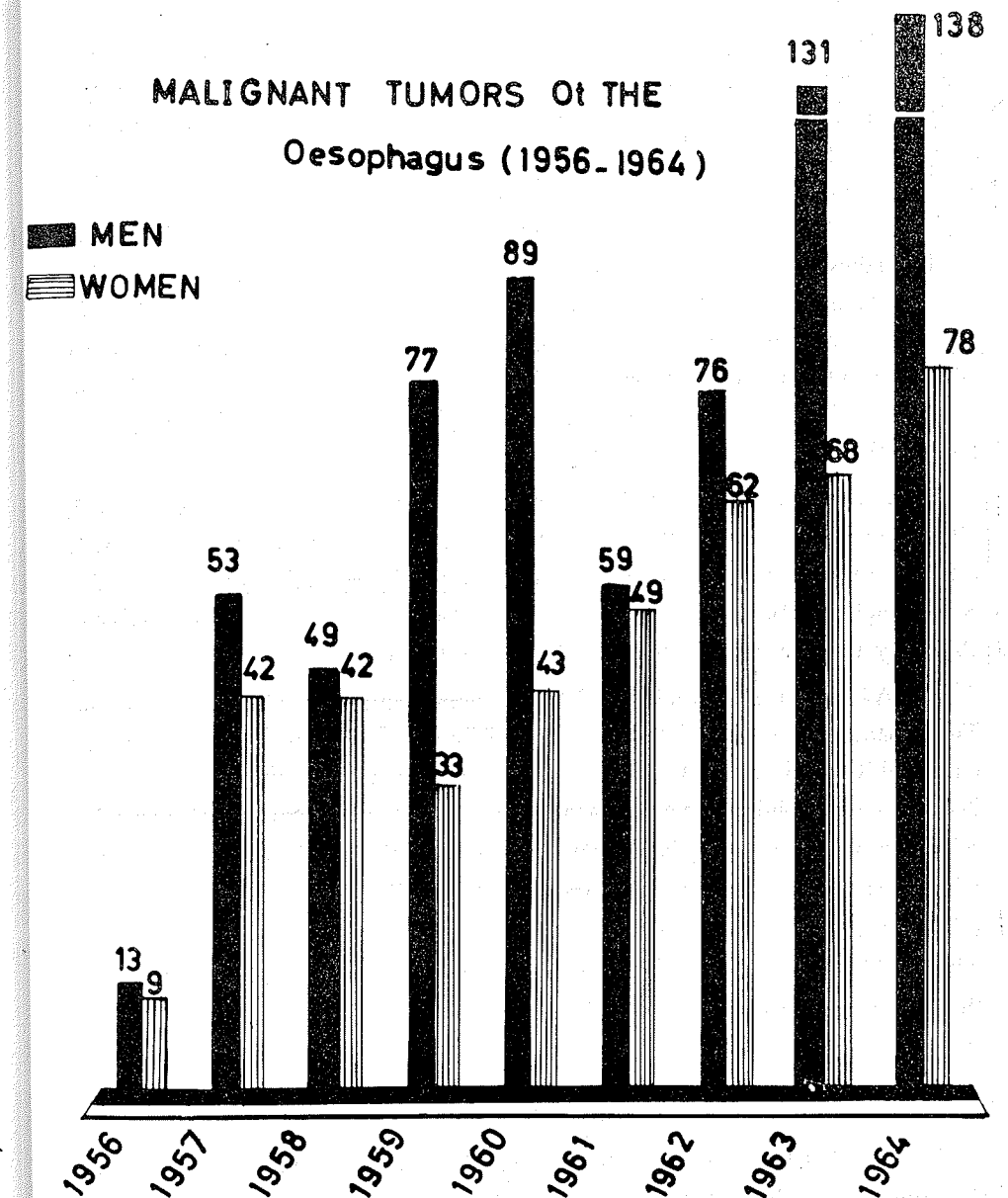
In addition to Roffo and Chikomatsu a lot of investigators have done many long term experiments on the carcinogenic effects of tobacco on the skin in which most of them got positive results. (9. 11. 12. 13. 14. 15. 19. 38. 39.).

The carcinogenic effects of the condensed smoke of cigarettes on the skin has been proved. Particularly the investigations of Cuzin and Guérin in 1957 and 1958 confirm this, but none of the above mentioned results can prove that there is any relationship between tobacco and cancer of the lung or esophagus. However in the following epidemiological studies the relationship of cancer of the lung and cigarette smoking became evident (3. 4. 28. 34. 38. 39.). In addition experiments done on animals confirmed this conceptum.

From the above mentioned experiments, one can conclude that there is some relation between the production of cancer and the dosage of consumed tobacco.

Epidemiological studies have shown that in many parts of the world some kinds of cancer is more prominent than the others. One of these is cancer of the esophagus, which is very frequent in some parts of the world and this can be seen in the epidemiological reports of W. H. O. (35).

Southern Africa, Mexico and Central Asia are some of the areas in which cancer of the esophagus is very common. It seems that some of the most important causes of cancer of the esophagus is the social habits and the way of living of the people of these areas. By paying more attention we will see that most of these people have some kind of living



habits which are similar to each other. For example the consumption of hot foods and unsaturated fat, the way of consumption of tobacco, etc.

Kolycheva (1962, 1963) demonstrated in the areas of Kazaghestan and Gooryev in U. S. S. R. (near to the Caspian sea and the shores of volga and Ural Rivers) that cancer of digestive tract especially the esophagus were more frequent than other kinds. Particularly cancers of the esophagus were the most common in the Gooryev area. (The percentage of this cancer is 60%). Most of the patients are women, of younger age group. In Kazaghestan the frequency of cancer of the esophagus is 14%. Possibly the habits of these people such as the consumption of larg quantities of fish, hot food and Nass can be considered some of the causes of cancer of the esophagus. Histological studies in this area showed other lesions such as leukoplasia, esophagitis and atrophic processes which may have some effect in the production of cancer.

According to the epidemiological investigations of Kolycheva (1962, 1963) cancer of the digestive tract is frequent in the areas of Kazaghestan and Gooryev, and cancers of the esophagus are the main types. The habit of having hot foods especially hot tea is outstanding among these people. Probably the soil plays a great role in the frequency of these cancer.

As it was mentioned before in many parts of Central Asia and Turkamansahra it is the habit of the people to consume NASS by chewing it. The majority of these people are peasants who prepare NASS by combining tobacco, ash, lime and a small quantity of vegetable oil. It seems that there is a relationship between the frequency of cancers of the esophagus and the consumption of NASS in these people, since the major part of the NASS combination is tobacco. Meanwhile in these parts where NASS or tobacco is being consumed the frequency of cancer of the throat and esophagus is high.

Experimental Research

The following investigations were based on the effects of NASS on the squamous epithelial layer of the esophagus in rats and Mice as well as its effects on the skin of Rats.

Materials And Methods

The material which we have used in our experiments is a 5% aqueous solution of NASS and was prepared by the following method:

A 5% aqueous solution of NASS and was put in a temperature of 37° to 40° C., and mixed for two hours. Then this solution, incubated at 40° C., over a 24 hours period was used after being filtered twice.

NASS contains the following substances and the portions of these materials are adapted according to investigations of the author in the area.

Tobacco powder (autumnal crop) 1000-1500 Grs.

Wood-Ash 200-300 Grs.

Lime 50-100 Grs. per Kg. of Mixture

To this a little water is added 20- 50 Grs. » » » »

To each kg. of this mixture 150-200 Grs. of vegetable oil (cotton-seed or sesame).

Materials And Methods

The animals selected for our experiments were Ratus ratus Norvegicus albinos and (DBA_r X O₂₀) F₂ mice. The total number of the animals used, from both sexes was 120 (70 Rats and 50 mice). The substance was used in two ways, one by rubbing it on the skin and the second by inserting it in the stomach by means of a stomach tube.

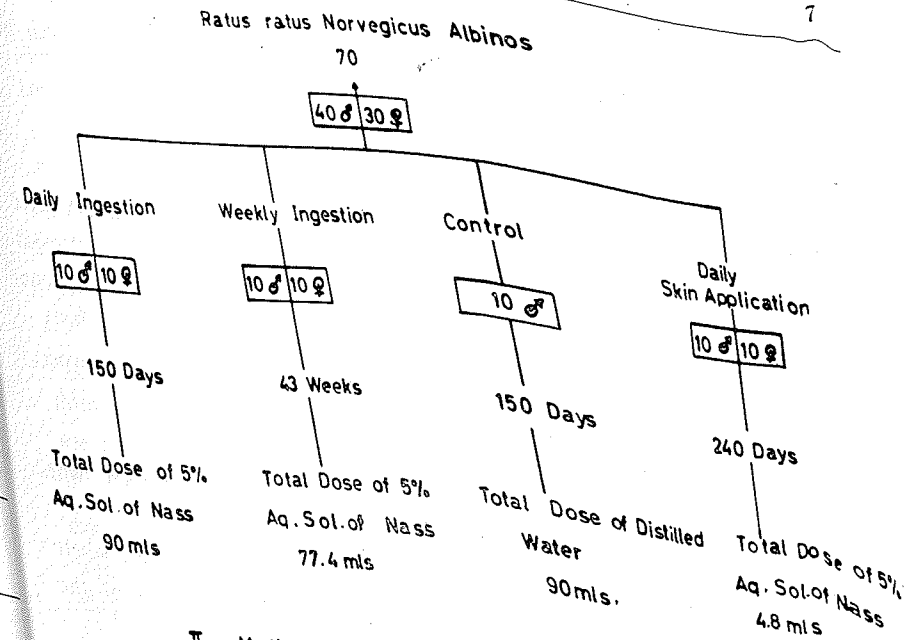
This stomach tube consisted of a blunt needle with a round ball of silver at the end in which a hole was made to allow the fluid to escape. The needle was connected to a syring.

We divided our animals in four groups:

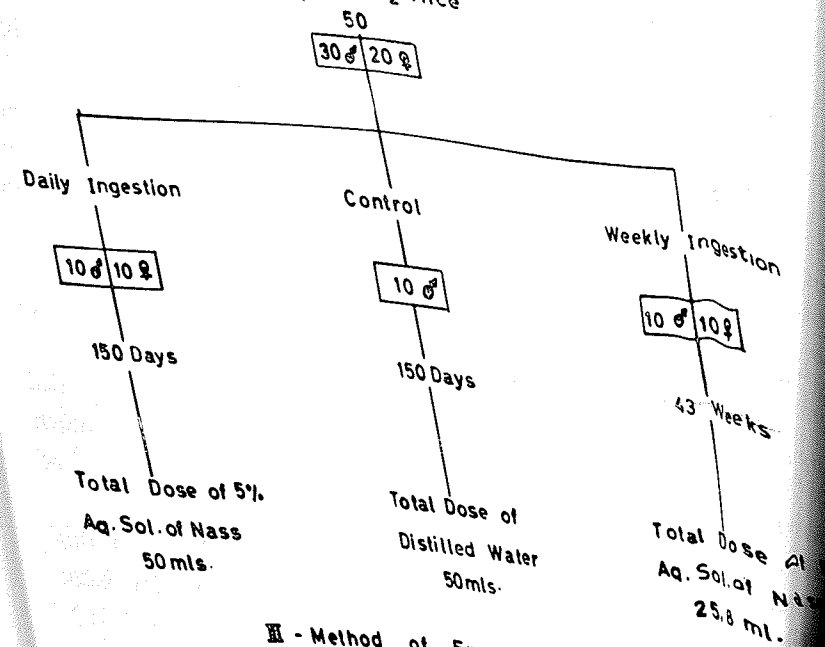
- 1- First group consisted of 20 Rats and 20 mice to which we gave the substance orally, every day, for 150 days. It was given by means of the stomach tube.
- 2- Second group consisted of 20 rats and 20 mice to which we administered NASS orally, once a week, for 43 weeks. It also was given by means of the stomach tube.
- 3- Third group consisted of only 20 rats and we rubbed the substance on the skin of the posterior part of the back daily for 240 days.

Substance Dosage Per Ingestion or Application	Species of Animals	Age of Animals in the Beginning of experiment	Kind of Application	Number of Ingestions or Skin Applications	Total Dose Per Animal	Sex & Total no of Animals
0.6 ml. of 5% Aqueous Solution of Nass	Ratus ratus Norvegicus Albinos	10 months	Stomach Tube Daily	150 Times	90 ml	10 ♂ 10 ♀
0.6 ml Distilled Water	Ratus ratus Norvegicus Albinos	10 months	Daily with stomach tube	150 Times	90 ml	10 ♂ 10 ♀
0.2 ml of 5% Aqueous Solution	(DBA ₁ × 020)F ₂ Mice	4 months	Daily with stomach tube	150 Times	50 ml	10 ♂ 10 ♀
0.2 ml Distilled water	(DBA ₁ × 020)F ₂ Mice	4 months	Daily With stomach tube	150 Times	50 ml	10 ♂ 10 ♀
1.8 ml of 5% Aqueous Solution of Nass	Ratus ratus Norvegicus Albinos	10 months	weekly with stomach tube	43 Times	77.4 ml	10 ♂ 10 ♀
0.6 ml of 5% Aqueous Solution of Nass	(DBA ₁ × 020)F ₂ Mice	4 months	weekly with stomach tube	43 Times	25.8 ml	10 ♂ 10 ♀
0.2 ml of 5% Aqueous Solution of Nass	Ratus ratus Norvegicus Albinos	10 months	Daily skin Application	240 Times	48 ml	10 ♂ 10 ♀

I - Material & Method of Experimentation



II - Method of Experimentation in Rats .
(DBA₁ × 020)F₂ Mice

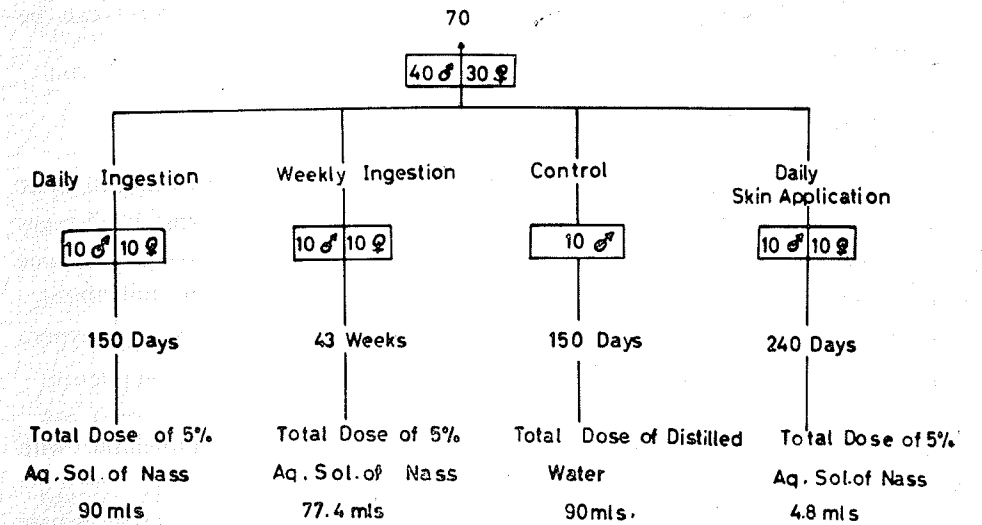


III - Method of Experimentation in Mice .

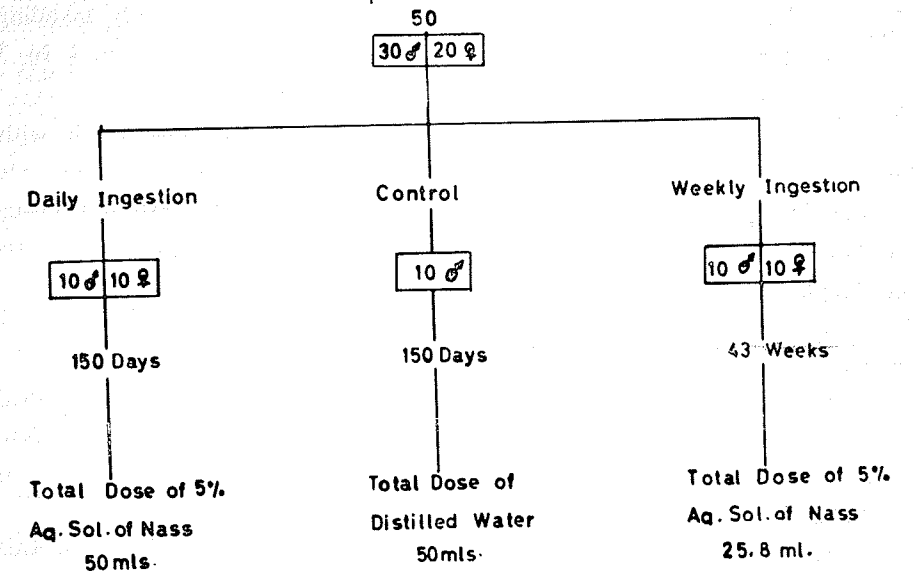
Substance Dosage Per Ingestion or Application	Species of Animals	Age of Animals in the Beginning of experiment	Kind of Application	Number of Ingestions or Skin Applications	Total Dose Per Animal	Sex & Total no of Animals
0.5 ml. of 5% Aqueous Solution of Nass	Ratus ratus Norvegicus Albinos	10 months	Stomach Tube Daily	150 Times	90 ml	10 ♂ 10 ♀
0.6 ml Distilled Water	Ratus ratus Norvegicus Albinos	10 months	Daily with stomach tube	150 Times	90 ml	10 ♂
0.2 ml of 5% Aqueous Solution	(DBA ₁ × O ₂₀) _{F2} Mice	4 months	Daily with stomach tube	150 Times	50 ml	10 ♂ 10 ♀
0.2 ml Distilled water	(DBA ₁ × O ₂₀) _{F2} Mice	4 months	Daily With stomach tube	150 Times	50 ml	10 ♂
1.8 ml of 5% Aqueous Solution of Nass	Ratus ratus Norvegicus Albinos	10 months	weekly with stomach tube	43 Times	77.4 ml	10 ♂ 10 ♀
0.6 ml of 5% Aqueous Solution of Nass	(DBA ₁ × O ₂₀) _{F2} Mice	4 months	weekly with stomach tube	43 Times	25.8 ml	10 ♂ 10 ♀
0.2 ml of 5% Aqueous Solution of Nass	Ratus ratus Norvegicus Albinos	10 months	Daily skin Application	240 Times	48 ml	10 ♂ 10 ♀

I - Material & Method of Experimentation

Ratus ratus Norvegicus Albinos



II - Method of Experimentation in Rats .

(DBA₁ × O₂₀)_{F2} Mice

III - Method of Experimentation in Mice .

4- Fourth group consisted of 10 rats and 10 mice as control.

The dose and other data, in detail, used in this experiment can be found in tables: 1, 2, and 3.

Histological Findings.

First group: The first specimen in rats was taken two month after the commencement of the experiment. In this period hyperplastic changes together with a slight increase of the basal layers was obvious (in one rat from 5 cases following autopsy).

In the specimens taken in the third and fourth months the hyperplasia of squamous layers was distinct.

The most important changes in this period were:

- 1- Acanthosis of surface of stratified squamous epithelium with proliferations of the basal cells.
- 2- The more important changes were seen, in some areas, where there were some basal cell proliferation with formation of the rete ridges.
- 3- In some other areas, the changes were even more severe where basal cells became pleomorphic and hyperchromatic focally invading the underlying stroma by rupturing the basal membran. (In 3 out of 5 rats).

In addition to polymorphism, rupture of the basal layer along with infiltration of the submucosa and the presence of neoplastic cells were quite obvious in the specimens taken at the end of the fourth month and during the 5th month. All of these changes were mainly seen in the lower parts the esophagus and in the forestomach. (In 2 out of 6 rats).

In 15 mice examined up to the 3th and 4th months no obvious changes were seen except slight hyperplasia. (In 6 out of 15 mice).

Of the first group of 20 rats used during the experiment, two died within the first month, On in the second month and another in the fourth month Five mice also died during the experiment because of rupture of the esophagus.

second group: In 12 rats which have been examined up to this date, only three have shown hyperplastic changes. None of the mice have shown any noticeable abnormalities.

Third group: The skin of only ten rats were examined, and four showed loss of sebaceous glands in the area of experimentation, but we have observed no other changes.

Fourth group: Controls. There are no obvious changes.

Discussion

Carcinoma of esophagus and the effect of ingested food on its production has been a matter of great contraversary debate.

As the investigation of Wahi in 1963 shows cancer of the mouth, throat and esophagus are very common in the people of central Asia who are consuming NASS by means of chewing.

From the epidemiological studies and statistics on hand it seems that cancers of the esophagus are outstanding in the North Eastern parts of Iran because most of the sufferers belong to this area and the people of this area are acquainted with the frequency of this disease. The other important point is that this area is located next to the area of Kazaghestan and Gooryev.

It seems that the following points have some effects on cancers of the esophagus.

- 1- Consumption of NASS by means of chewing a great quantity.
- 2- Consumption of hot foods and tea.
- 3 - Some Ratial factors.
- 4 - The soil.

Our studies on the effect of NASS on the squamous epithelium of the esophagus have shown changes from slight hyperplasia to early neoplastic changes .

We have also found changes due to the application of NASS on the skin of rats which are the same results as those of the invistigations of Guérin and Cuzin. Therefore it seams that possibly NASS has some effect on the production of cancers in Turkamansahra.

It would be of great interest to us to find out which is the carcinogenic element of NASS. Is it tobacco (of which there is more probability of being so) or are the other factors, such as lime and vegetable oils, the

cancer producing materials? In order to find an answer to all these questions there are several experiments being conducted now.

Summary

Our experiments were based on the effects of NASS on squamous epithelium layer of the esophagus in rats and mice as well as its effects on the skin of rats. These experiments concluded hyperplastic and early neoplastic changes in esophagus of some rats and loss of sebaceous glands in the skin of rats.

Résumé

Nos expériences ont été basées sur les effets de NASS sur l'épithélium malpighien de l'oesophage sur les rats et les souris, ainsi que l'effet de NASS sur la peau des rats. Ces expériences ont conclu à une hyperplasie et des changements néoplasiques précoces de l'épithélium oesophagien de certains rats et une atrophie des glandes sébacées de la peau des rats.

LEGENDE FOR FIGURES

- Fig. 1 Shows section of esophagus from a white rat treated with NASS for 2 months. The Section shows hyperplasia of squamous epithelium and proliferation of basal layer.
- Fig. 2 Shows section of esophagus from a white rat treated with NASS for 3 months. The Section shows proliferation of the rete pegs and polymorphonuclear infiltration of the basal layer.
- Fig. 3 Shows section of esophagus from a white rat treated with NASS for 4 months. The section shows the abnormal increase mitotic activity in the basal layer,
- Fig. 4 Shows section of esophagus from a white rat treated with NASS for 4 months. The section shows early neoplastic changes which has broken through the basement membrane into submucosal tissue.
- Fig. 5 Shows section of esophagus from a white rat treated with NASS for 5 months. The section shows presence of infiltrating early neoplastic cells in the submucosal layer.
- Fig. 6 Shows section of esophagus from a white rat treated with NASS for 5 months. The section shows early neoplastic cells infiltrating

the musculare of the esophagus.

- Fig. 7 Section of normal rat skin with sebaceous glands and hair follicules.
- Fig. 8 Shows section of rat skin after local application of NASS for 2-3 months. The section shows beginning atrophy of sebaceous glands.
- Fig. 9 Shows section of rat skin after local application of NASS for 5 months. The section shows complete atrophy of sebaceous glands.

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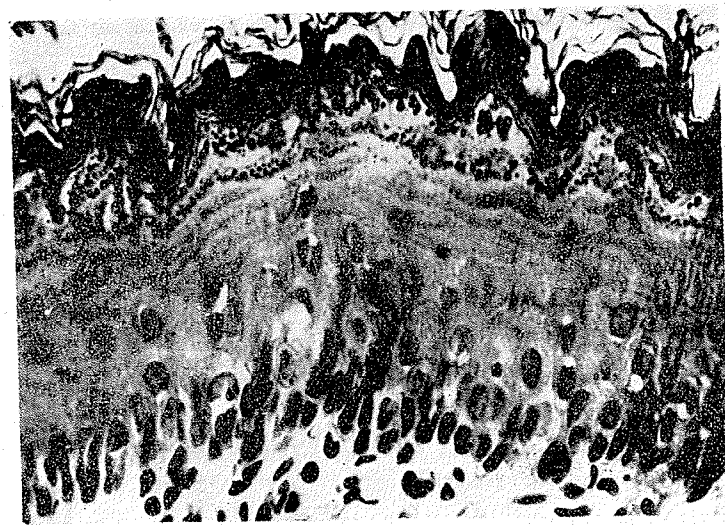


Fig. 1

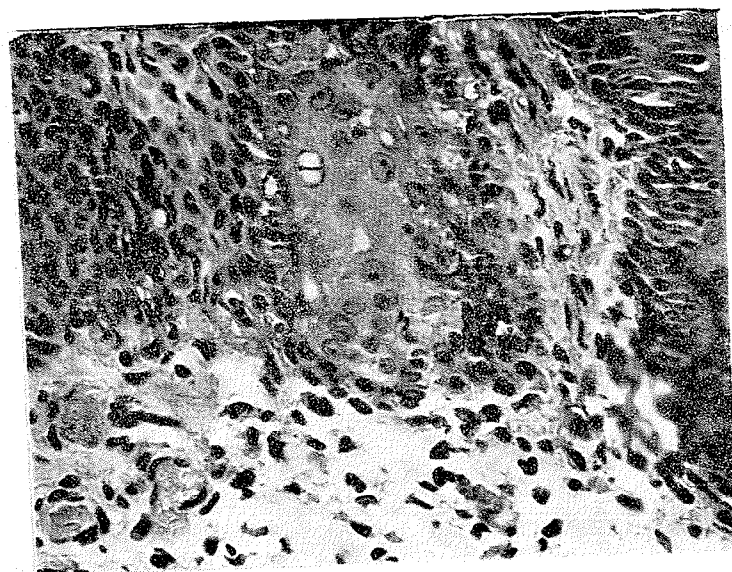


Fig. 2

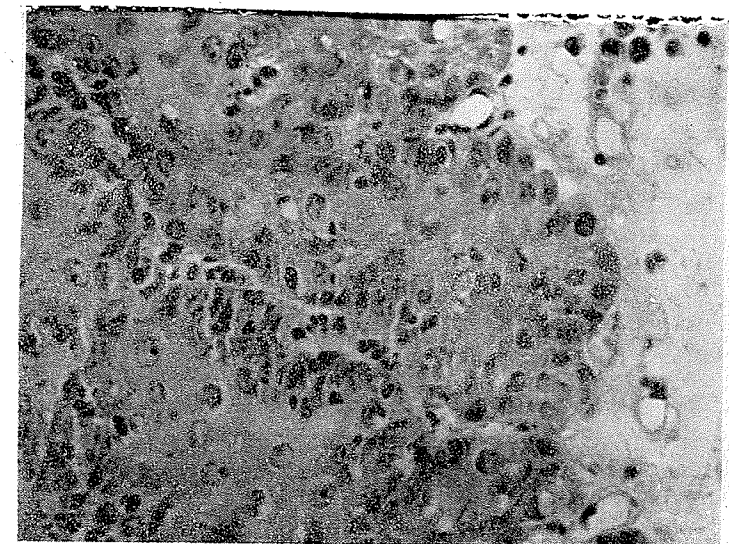


Fig. 3

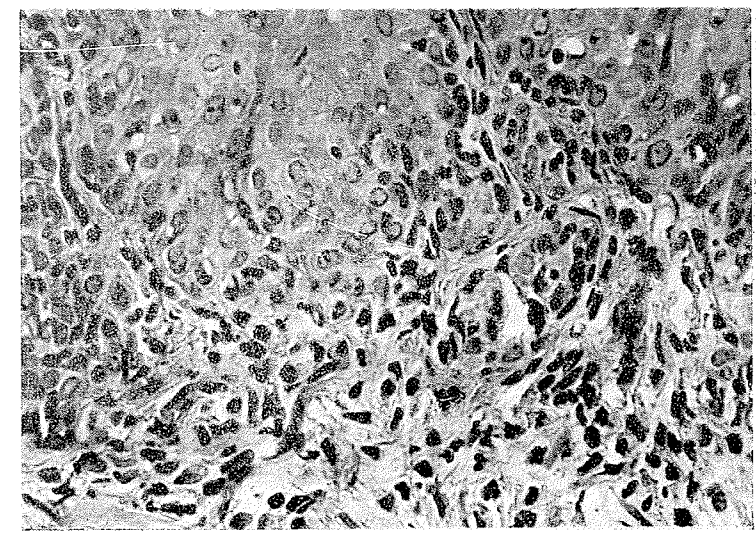


Fig. 4

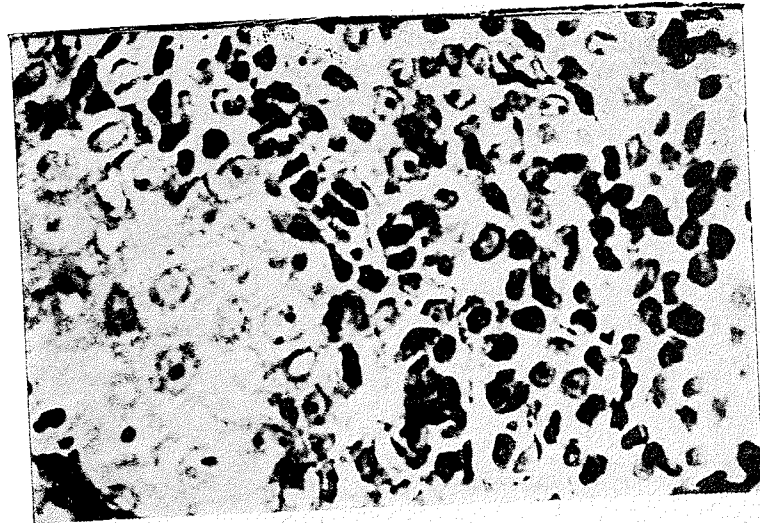


Fig. 5

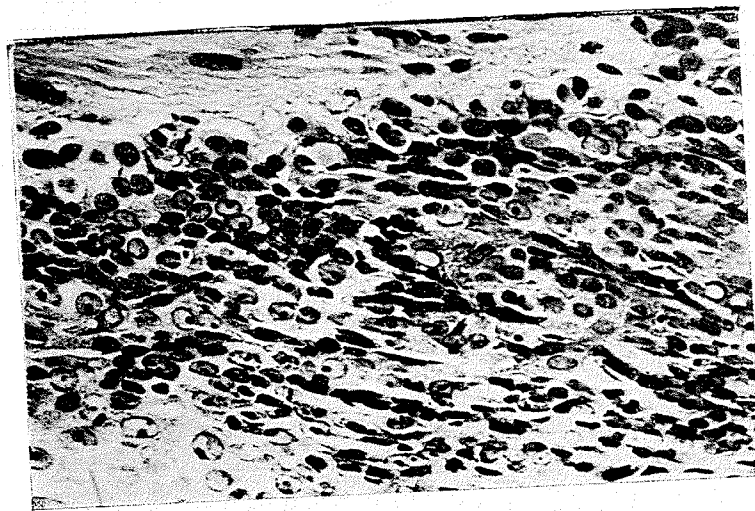


Fig. 6

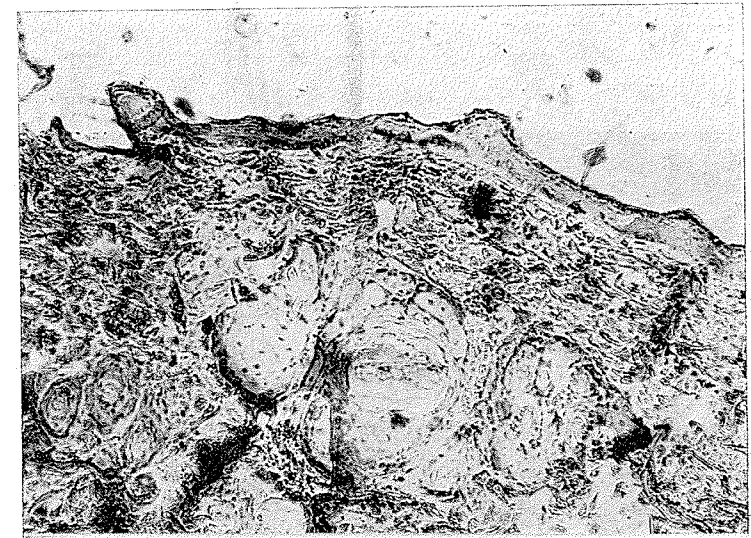


Fig. 7

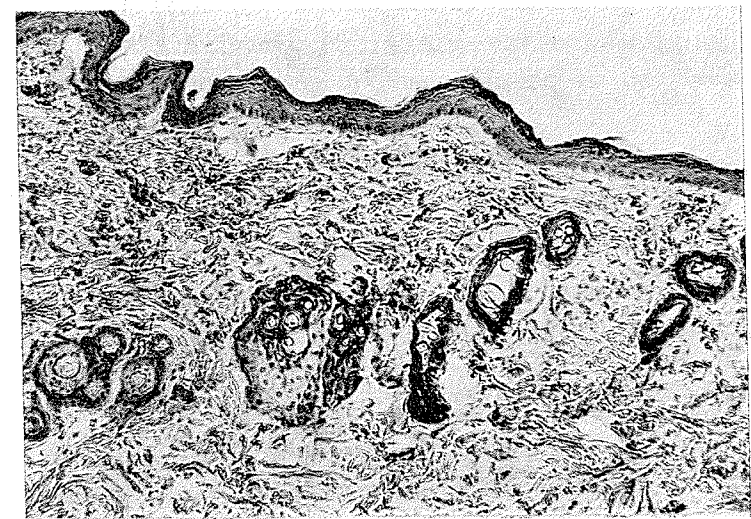


Fig. 8

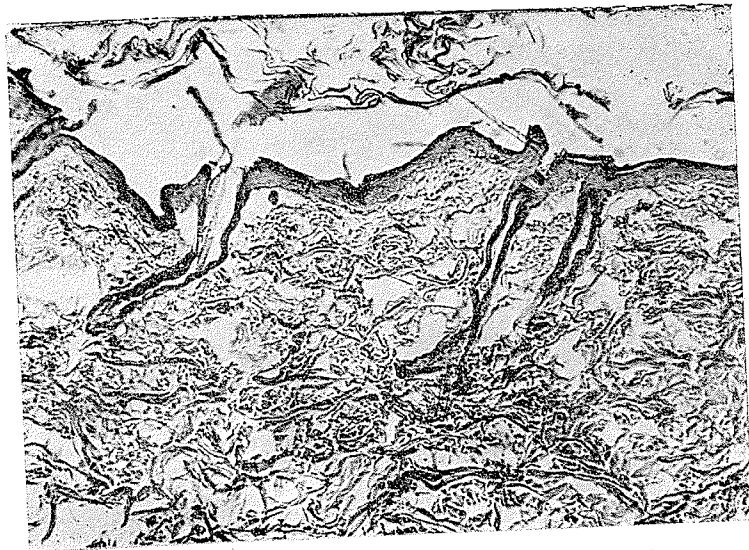


Fig. 9

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CANCER SITUATION IN IRAN

A SURVEY OF THE MOST FREQUENT FORMS AND SITES AND THE COMPARISON OF THE PREVALENCE WITH SOME OTHER STATISTICS

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Cancer survey, if it is considered as based on histological diagnosis of this disease, is not very long-standing in Iran.

The first pathology laboratory was created by Ministry of Public Health in 1937 in Tehran. In 1939 it was transferred, together with the hospitals of the Ministry of Public Health, to the Tehran Medical School.

This Laboratory was splendidly developed by the lamented Professor MOSTAPHA HABIBI, who made it into a first class centre.

It was only later, some time after the foundation of this laboratory, that certain Health Organizations devoted themselves especially to the case finding, diagnosis and treatment of cancerous patients in Tehran and the Cancer Institute was created.

The first step in controlling cancer is to have a complete and accurate knowledge of its occurrence, the most common forms, and the various factors which are conducive to their formation.

In countries like Iran where, as yet, there is no cancer control programme, the carrying out of the above preliminary steps is subject to two main limitations :

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