THE SELECTED ACADEMIC THESES ON NASOPHARYNGEAL CARCINOMA

鼻咽癌論文選集

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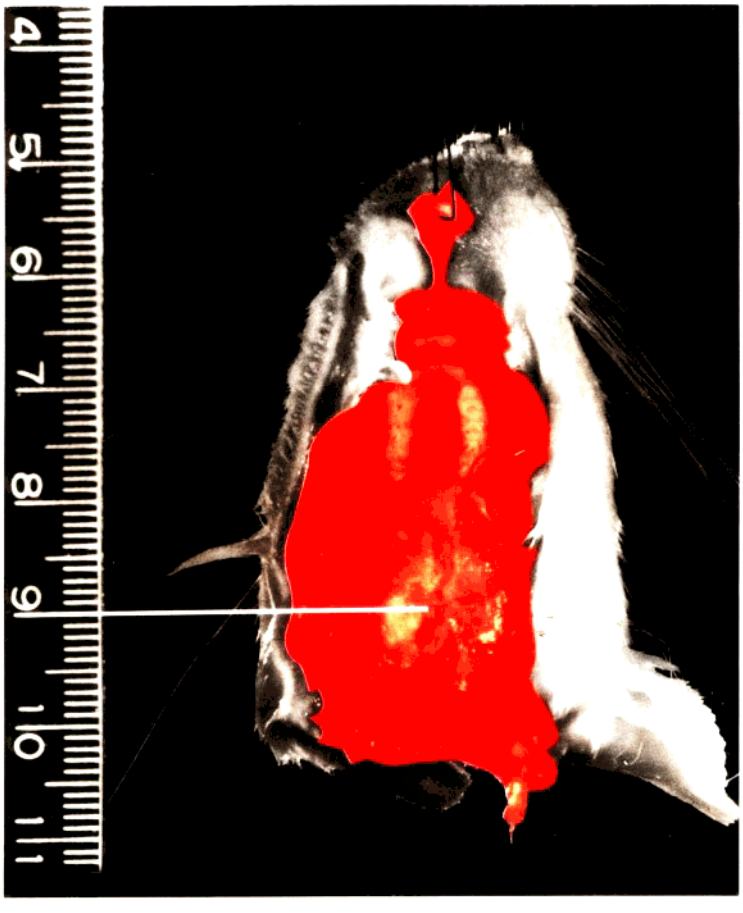

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INDUCTION OF NASOPHARYNGEAL CARCINOMAS IN A LBINO RATS BY DIETHYLNITROSAMINE(DEN) AND DIMETHYLBENZ(A, I)ANTHRACENE(DMBA)

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Abstract: This experiment reported the induction of nasopharyngeal carcinomas(NPC) in albino rats by DEN(instillation once weekly for 18 successive weeks)and DMBA(insertion of loaded polyethylene tubes through the nostril into the nasopharyngeal cavity). The incidence of carcinoma in different groups were as follows: DEN group 63%; DMBA group 40%; DEN + DMBA group 59%; DEN + empty tube group 16%. Most of the tumors induced were squamous cell type, the histological appearance of these tumors resembled those observed in humans.

Masophryngeal cancer is geographically restricted to the Southeast Asia. In China the southern provinces are involved. Experiments done on animals to induce nasopharyngeal tumors are few. Ou tried to implant DMBA treated nasopharyngeal tissue subcutaneoously in bomologus mice and obtained squamous carcinomas. Wang obtained squamous cell tumors and columnar cell tumors of the nasal cavity and hard palate in mice by benzo(a)pyrene, methylcholanthrene and dimethylbenzanthracene.

This paper reports the work done in 1972-1974 by using DEN and/or DMBA to induce NPC (Nasopharyngeal Carcinomas) in rats.

MATERIALS AND METHODS

1 Chemicals:

DEN and DMBA were both synthesized by the Department of Chemistry of Hunan Medical university. The boiling pointof DEN is 172°-174℃ and the melting point of DMBA is 120℃.

Polyethylene tubes: Purchased from the market with an external diameter of 3mm, made spindle-shaped over flame, then 4mg of DMBA was inserted into each tube. The rounded end of the tubes were sealed on fire and the pointed end pricked with fine needles to make small holes on it, so that DMBA could leak out very slowly. Each tube was about 3.5cm in length.

DEN: 33.3% DEN was suspended in 1% $Tween_{40}$, freshly prepared before use.

Rats: Hybrid albino rats weighing 120-170 gms obtained from the college breeding room.

2 **Administration of carcinogenic substances:**

DMBA: DMBA loaded polyethylene tubes were inserted into the nasal canals of ether anaesthetized rats with its tip pointed to the nasopharyngeal cavity.

DNE: 33.3% DEN suspended in 1% $Tween_{40}$, 0.02ml(containing DEN 6.7mg)was directly instilled through the nostril to the nasopharyngeal cavity of ether anaecthetized rats by stumped needes once weekly.

3 **Grouping of animals: Albino rats were randomized into five groups and treated as follows:**

1)DMBA group: With loaded tubes inserted

2)DEN group: Instilltion of DEN once weekly

3)DEN + DMBA group: Loaded tubes inserted and DEN administered subcutaneously once weekly

4)DEN + Empty tubes group: Emlty polyetnylene tubes inserted and DEN administered subcutaneously once weekly.

5)Control group: Fed under normal conditions

Postmortem examinations were performed on animals that died after 30 days of treatment, those that died within 30 days were discarded. A total of 133 rats were examined.

Most of the animals died spontaneously after treatment, except 28 survivors that were not killed until the end of the 40th week. Control rats were sacrified at different intervals.

The whole length of the nasopharyngeal canals including the basal portion of the skull were carefully sectioned out and fixed in 10% Formalin; decalcified with 5% Nitric Acid; embedded with paraffin and 5 micron serial sections were made. All sections were routinely stained with hematoxilin and eosin, some with Gomori stain also.

RESULTS

1. General conditions of the treeated animals:

All animals became very feeble and liable to infection after tube insertion. They might have died if antibiotics were not used in time, within the first two weeks of insertion of the tbues.

The animals receiving DEN became feeble, with pilo-erection and hypomotility and some showed difficulty in respiration and bleeding from the nasal cavities.

Attempts were made to increase the dosage of DEN from 6.7mg to 10mg from the 13th week, but some animals suffered from convulsions and respiratory arrest so DEN was discontinued from the 18th week onwards. The accumulative dose of DEN for each rat was 126-150mg.

2. The pathological changes and incidences of carcinoma formation in each group of rats:

The nasopharyeal epithelia of the 10 controlled rats were essentially normal, except one case with metaplasia of squamous cell epithelium and a few with mild degree of metaplasia and infection. None developed tumors.

On the other hand, in the carcinoma inducing groups, the findings varied from congestion of the soft palate; small elevations of 1-2mm in diameter in the nasopharyngeal cavity under the soft

palatrlpartial to complete obstruction of the nasopharyngeal cavities of thickening of the whole nasopharyngea cavity and tubercular nodules of about lmm in diameter at the esophageal end of the nasopharyngeal cavity.

The pathological changes and incidences of carcinoma formation of each group are outlined on Table 1.

Table 1. Grouping of animals, pathological changes and incidences of carcinoma formation

Groupping	Carcinogens used	Route of administration	Number of animals examined	Pathological changes of the nasopharyngeal portion				Number of tumors found	Incidence of NPC (%)	Notes
				Precancerous changes	Types of NPC observed*					
					In situ	Early invasive	Infiltrative			
I	DMBA	Insertion of loaded tubes	25(1) 20(2)	5 7	4	7 5	2	13 5	52% 25% 40%	(1)dead (2)killed
II	DEN	Instillation	27	6	5	9	3	17	63%	3 killed
III	DEN+DMBA	Subcutaneous injection Insertion of loaded tubes	27	6	3	9	4	16	59%	1 killed
IV	DEN+Empty tubes	Subcutaneous injection	24	5		2	2	4	16%	4 killed
V	None	-	10							

* If more than one types of tumor were found in the same animal the most malignant one was counted.

Microscopic findings: Among the 55 tumor-bearing animals 73 tumors were observed. Some were localized and some were multiform with different degrees of malignancy such as carcinoma in situ, early invasive, infiltrative to extensive invasive forms.

As to the site of NPC in the nasopharyngeal cavity, in DMBA group most tumors were located in the basal portion of the nasopharynx at the postedior third of the soft palate, while in DEN group, the pathological changes occured more frequently on the lateral walls of the nasopharyngeal cavity in the epithelium of the posterior third of the soft palate.

Histologica feactures of the tumor cells: Most of them were squamous cell-type, either well differentiated, poorly differentiated or undifferentiated(but few in number). One case waas a kerato-adenocarcinoma, No sarcomas were seen either in the naso phsoparyngeal portion or subcutaneouly.

The histological characteristics of the tumors could be divided into three categories: Carcinoma in situ, early invasive tumors and infiltrative type. The findings were as follows:

(1)Carcinoma in situ: Among the 73 tumors 23 were in situ. The moderately thickened epithelial cells of the nasopharynx underwent metaplastic changes and formed stratified squamous epithelial cells, where most of the tumors originated from.

The particular factrues of these tumors were: the nuclei were somewhat larger and hyperchromatic; number of mitosis increased; the cells were irregular in size and shape; lack of polarity but

the basement membrane was intact. The cells were crowded together.

Sometimes tumors of this type might have originated directly from metaplastic or dysplastic epithelia.

(2)Early invasive tumors: 39 early invasive tumors were observed, the mode of growth was multiform. Chiefly.

1) Early invasive tumors originated from *carcinoma in situ*, the tumor cells spread along the ducts or salivary glands across the basement membrane into the adjacent tissue and penetrating the basement membrane directly. 2)Arising from the basal cell layer, those cells underwent metaplastic changes and formed tumor nests in the stroma. If the tumors originated from the folds of the epithelium of the mucosa an umbilicus-like protrution might be formed. Some early invasive tumors were of spherical form and others were of multiple-layer type or formed square-shaped nests(Fig. 1). 3)Sometimes there were papillary hyperplastic changes of the naspharyngeal epithelia forming papilloma or epidermoid carcinoma. The malignancy occurred most frequently either on the top portion or the basal portion of the nasopharyngeal epithelium.

(3) Infiltrative tumors: All together 11 infiltrative tumore were found. Typical tumor nests spreaded into the stroma. Microscopically the tumors might be divided into three categories: Squamous cell, undiferentiated and adenokeratinized types. Squamous cell type was the major form found, the nests of the tumors were spherical, rod form or fusiform sometimes the keratinized epithelial pearls might be encapsulated by tumor cells.

The tumor nests might spread extensively into the interstitial tissue. One case showed extensive intramuscular invasion, lymphatic embolism (Fig. 3.) and pulmonary metastasis (Fig. 2.). Some of the squamous cell tumors, infiltrating a large part of the nasopharyngeal mucosa(Fig. 4, 5.)looked like a mushroom and protruded into the nasopharyngeai cavity. Some tumors were formed from spindle-shaped columnar cells. Few were undifferentiated form and onw case was a keratoadenocarcinoma(Fig. 6.)

3. The changes of the interstitial tissue:

The interstitial tissue surrounding the nests of tumor changed markedly. Hyperplasia of reticulo-fibrous tissue was present, the ground substances stained more eosinophilic and infiltration of lymphocytes and plasma cells in the stroma was marked. The secretion of the nasopharyngeal glands increased first, then the glands underwent metamorphotic changes and some of the glandular epithelial cells underwent metaplastic and/or malignant changes.

The pathological characteristics of the rat tumors obtained in our experiment closely resembled those of human beings. Human nasopharyngeal cancers were mostly of poorly differentiated squamous cell type, a few were undifferentiated.

In our experimentally induced nasopharyngeal tumors of the rats, the cells were either well differentiated or poorly differentiated, only few were undifferentiated. And there were different degrees of keratinization of the well differentiated cells. The poorly differentiated cells were of multiform type with small oval or spindleshaped nests. These histological changes resembled those observed in human NPC.

Some tumors in our experimental model resembled columnar cell tumors and showed irregularity of glandular arrangement. No lympho-epitheliomas were observed.

4. Incidence and time relation of tumor formation.

Among the 123 treated animals observed in our experiment, 55 rats developed 73 tumors, in 35 animals the tumors appeared within the first 24 weeks of administration of carcinogenic substances. The incidence of carcinoma formation was 63%. The following table shows the incidences of carcinoma formation of different groups within 24 weeks.

Table 2. Incidences of carcinoma formation in different groups within 24 weeks.

Grouping	Animals examined	Number of tumor -beaing animals	Nuber of tumors formed within 24 weeks	Incidence of carcinoma formation within 24 weeks
DMBA	(1)25 (2)20	13 5	11	48%
DEN	27	17	8	30%
DEN + DMBA	27	16	14	51%
DEN + Empty tubes	24	4	2	8%

(1)died spontaneously

(2)killed

In DMBA group 25 animals died, the polyethylene tubes were left in situ in 18 of them, 13/18 were tumor-bearing, and the loaded DMBA had partially leaked out. Incidence of carcinoma formation was 52%. Among the 18 with tubes in situ, 4 tubes were twisted but only 1/4 developed nasopharyngeal tumor. As for the 20 killed animals only one tbule was letf in situ, 5 animals had nasopharyngeal tumors, the incidence was only 25%. Obviously the low incidence was due to tube displacement.

In the DEN group three were killed, all had nasophtaryngeal tumors. As to the time of tumor formation. In DEN group the earliest one was a carcinoma in situ, 58 days after treatment. The first infiltrative tumor was observed 145 days after treatment in DEN + DMBA group. This tumor not only involved the interstitial tissue, but also muscular invasion, lymphatic embolism and pulmonary metastasis were observed.

In DEN instillation group, some rats developed hepatoma in addition to NPC and a large number of the rats were found to have parasitic cysts in the liver. These conditions might have contributed to the death of the animals.

In short, in our experiment, we observed nasopharyngeal carcinomas within a short period and the histological feaures of the tumors induced closely resembled those observed in human beings. Insertion of the tubes might have traumatized the nasopharynx and made the condition more complicated.

DISCUSSION

A lot, of experimental reports have focused on the induction of hepatic and esophageal tumors in animals by nitroso compounds[3], but none referred to inducing tumors in the nasopharynx, only tumors of the nasal cavity were reported[4][5].

In our experiment, instillating DEN into the nasopharyngeal cavities of rats to induce tumors, demonstrated an incidence of tumor formation as high as 63%, which was the highest among other groups, indicating that the nasopharynx of rats is sensitive to nitroso compounds. But when DEN was administered subcutaneously the incidence was only 16%, showing that the local concentration of DEN is an important factor in tumor formation. Besides we could not neglect the influence of irritation of the polyethylene tubes to the nasopharynx as seen in DEN + DMBA group. The high incidence(59%) of tumor formation in DEN + DMBA group indicated a synergic action of the two carcinogenic substances used. The same result was observed by others. Herrold reported that when 3, 4 benz(a)pyrene was disolved in $Tween_{60}$ or olive oil the incidence of lung tumors induced in hamaters was different. In our experiment we used 1% $Tween_{40}$ as a solvent for DEN. It facilitated the adsorption of the carcinogenic substance to the nasopharyngeal epithelia and also avoided inspiration of oil into the lung which might cause aspiratory pneumonia.

It was reported in the literature that the nasopharynx of rats is insensitive to polycyclic aromatic hydrocabbons and suggested that hamsters should be used. Considering that the epithelia of nasopharynx of rats is more similar to that of human beings and easier to be obtained, we decied to use rats. Through our experiments we found out that the most important point was long and uninterrupted adminitration of small dosages of carcinogenic substances to the nasopharyngael epithelia, and there were no species difference. For instance in DMBA intubation group, among 59 animals 25 died spontaneously, and 18 tubes were left in situ, the incidence of tumor formation was 52%, while the 20 killed rats only one tube remained in place and the incidence of tumor formation was only 25%, or course intubation and instillation might have traumatized the mucosa and caused infection, this made the conditions more complicated for etiological analysis.

Our report showed that treated rat nasopharygeal epithelia underwent squamous cell metaplasia, metamorphosis and the formation of carcinoma in situ, early invasive or infiltrative tumors. The pathological characteristics of induced tumors resembled those observed in early stages of induced esophageal tumors of rats by nitroso compounds. But the mucosa of nasopharynx had its own peculiarities, so that if the tumors arose directly form the columnar epithelial cells the appearance of the tumors would have been different.

Our experience showed that this model is useful for the study of the carcinogensis of NPC formation.

The pathogenesis of NPC in humanbeing is a complicated problem. According to a survey on its pathogenesis, it was reported that benzo(a) pyrene and nitroso compounds were found in the homes of NPC patients or they had a history of connection with chemical carcinogenic substances or coal tar etc.

At the international cancer conference some reports pointed out that there were direct relationships between bacterial infection, synthesis of nitroso compounds and cancer formation. It is important to elucidate the relationships between nitroso compounds and the formation of NPC.

The relationships between NPC and chemical, genetic, viral factors and the synergic actions of these factors needs further study.

ACKNOWLEDGEMENT

We are grateful to our colleagues Mrs You Jialu, Huang Jiliang and Wen Dongsheng for thir helpful suggestions and technical assistance.

用二乙基亚硝胺(DEN)、二甲基苯蒽(DMBA)诱发大鼠鼻咽癌的初步实验研究

本文报道用化学致癌物 DEN 及 DMBA 诱发大鼠鼻咽癌的实验研究结果，所用之化学致癌物，均系我院化学教研室合成，DEN 沸点为 170～174℃，DMBA 溶点为 120℃。

DEN 采用鼻咽部局部滴注或背部皮下注射二种途径给药。剂量均为每周一次，每次 6.7mg。临用时用 1% 吐温。新鲜配制成 33.3% DEN，每次 0.02ml。

DMBA 采用填有 4mg DMBA 晶体的自制塑料管从大鼠前鼻孔插入至鼻咽腔，并将鼻孔缝合以固定塑料管。

有的组还同时采用 DEN 及 DMBA 两种致癌物。

实验均用 120～170g 成年大鼠，从给药之日算起，30 日以后死亡的动物，均进行病理解剖，共检查 133 只大鼠，其中 28 只系第 40 周时杀死。对照组则分批杀死。切取整段鼻咽部，10% 福尔马林液固定，5% 硝酸液短期脱钙，石脑包埋，做连续切片，每片厚 5μm，苏木精一伊红染色。

实验结果见表 1。

表 1　实验动物分组及各组动物鼻咽部病变性质及发癌情况

组　别	给药途径	检查动物数	鼻咽部病理变化及发癌情况							注
			癌前变	原位癌	早癌	浸润癌	发癌数	发癌率		
DMBA 组	前鼻孔插管	25(1)	5	4	7	2	13	52%	40%	(1)自死
		20(2)	7	—	—	—	5	25%		(2)杀死
DEN 组	滴注鼻咽腔	27	6	5	9	3	17	63%		3 只杀死
DMBA + DEN 组	前鼻孔插管 皮下注射	27	6	3	9	4	16	59%		1 只杀死
DEN + 空塑料管组	皮下注射 前鼻孔插管	24	5		2	2	4	16%		4 只杀死
对照组		10	—	—	—	—	—	—		

所诱发的大鼠鼻咽癌有鳞癌、未分化癌及腺癌三种。主要为鳞癌，癌巢呈围瑰状、条索状，有时有角化珠，有时癌巢可呈囊状，癌巢由 2～3 层扁平的癌细胞构成，细胞核大，染色稍深，似鳞状上皮，囊内可有角化珠或角化片。1 例广泛浸润癌侵犯附近肌肉，淋巴管内形成癌栓并转移到肺。

有的鳞癌呈耸状或蕈状，突出鼻咽腔内甚至侵犯大部分鼻咽粘膜。有的癌肿成于梭形圆柱状细胞，有少数未分化癌及 1 例腺角化癌。

本实验所观察到的大鼠鼻咽粘膜经致癌物处理后有鳞状细胞化生、异型性以及呈显原位癌和早期鳞癌等病理改变，在病理形态学方面与临床所见早期鼻咽癌的病理变化接近。

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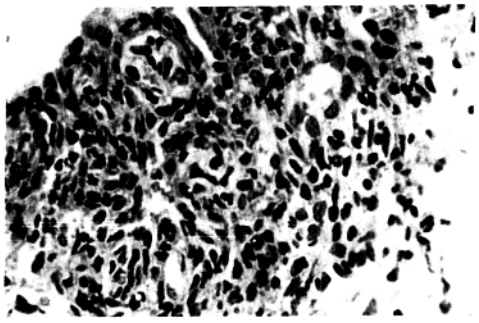

Fig. 1 Early invasive tumor: DEN + DMBA Group. Pathological No. 107, 145 days, 450 ×
The tumor cells were of oval, spindle or irregular from, forming a square nest.

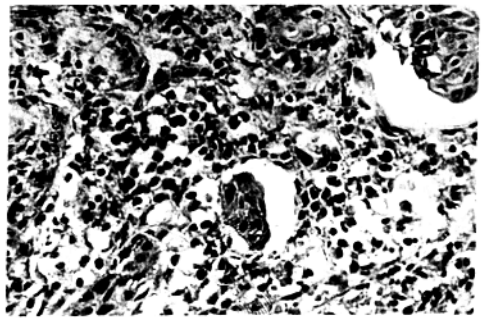

Fig. 3 Extensive invasive tumor: Pathological No. 109, 320 x
Note the lymphatic embolism

Fig. 2 Extrnsive invasive squamous carreinmoa: DEN + DMBA Group. Pathological No. 109, 154 days, 72 xThe tumor cells were small with large nuclei. The nests of the carcinoma were of rod spherical form and spreaded into the lateral walls of the nasopharyngeal cavity.

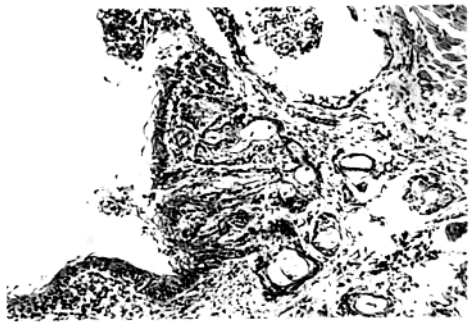

Fig. 4 Early invasive tumor; DMBA Group. Pathological No. 119, 207 days; 72 x
The tumor looked like a mushroom protruding into the nasopharyngeal cavity with the nests in the stroma.

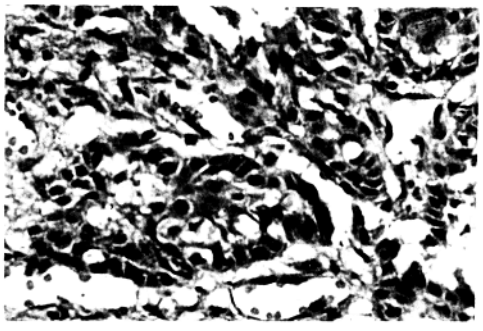

Fig. 5 Eacly invasive tumor; Pathological No. 119, 450 x

Note the nest of the carcinoma in the interstitial tissue

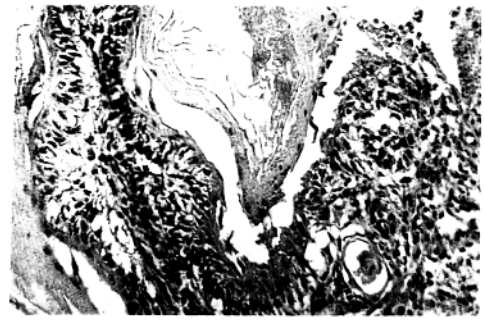

Fig. 6 Papillary carcinoma of the columnar epithelium; DEN Group. Pathological No. 134, 202 days; 240 x

The tumor cells were irregular in shape. Spreaded into the stroma. A part of the tumor looked like a papilloma.

INDUCTION OF NASOPHARYNGEAL CARCINOMA BY NITROSOCOMPOUNDS

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Abstract: This study confirmed using different kinds of nitrosocompounds, through different routes of administration may induce NPC in rats. The pathological lesions resembled closely to human NPC. Since dinitrosopiperazine induced considerable incidence of NPC without the induction of hepatoma, sugests that it may be a suitable agent to be used in the further study of NPC. Pathologic morphologic and histogenic studies have briefly described. The multicentric origin of the tumor growth is discussed in short. The organ – specificity and the relation of the nitrosocompounds to the etiological factors of human NPC might be the subjects for further investigation.

A number of nitrosocompounds have been used in the past to study nasal cavity tumors. In 1975 we reported the induction of nasopharyngeal carcinoma (NPC) in rats with diethylnitrosoamine (DEN) and/or dimethylbenzanthracene[1,7]. Hepatoma was incidentally noted to occur in the DEN group. Since then, on the basis of the former experiment, DEN and cyclic nitrosocompounds including nitrosomorpholine were used sepeatly to comfirm and improve The NPC model. Pathologic and histogenetic studies of the tumors were subsequently carried out. The NPC induced in rats resembled human NPC. was squamous carcinoma in nature but of well differentiated type.

MATERIAL AND METHODS

Chmicals

The nitrosomorpholin was synthesized by the chemical Department in the Medical College. the DEN and DNP were synthesized in our laboratory. Their boiling points, melting points and other chemical characteristics were determined.

Animals

Hybrid albino rats totalling 367, weighing 100 – 150 gms were obtained from the college breeding room and randomized to two groups: The first group consisted of 209 rats. Of these 179 rats received DEN and 30 rats served as normal controls. The second group, consisting of 158