FRACTIONATED INTRA-ARTERIAL CANCER CHEMOTHERAPY WITH METHYL BIS AMINE HYDROCHLORIDE; A PRELIMINARY REPORT*

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THE INTRAVENOUS INJECTION of Methyl Bis (B-Chloroethyl) Amine Hydrochloride§ has been found to be of clinical value in the treatment of the lymphomas,[6, 9, 20, 24] but of almost no value in the treatment of carcinomas.[14] The success of this treatment for malignant disease has been limited by the damage to the hematopoetic system.[17] Total body irradiation, which is comparable to intravenous HN_2 therapy, has also been used successfully for the lymphomas,[12] but has been of little value in the treatment of carcinomas.[19] Irradiation, however, is a more successful method of cancer therapy because it can be administered in fractional doses to localized tumors without damage to the bone marrow and other vital organs. If the effects of administration of HN_2 could be localized to the region of the tumor, it would be comparable to fractionated irradiation of local tumors, and effective treatment would be possible with minimal damage to normal tissues in other areas.

A method for accomplishing this type of therapy for regional carcinoma was suggested by the result of the accidental administration of HN_2 into the brachial artery of a patient with Hodgkin's disease. An erythema occurred which persisted several days and was followed by vesiculation and ulceration of the hand and forearm. Eventually the intense local reaction subsided and no irreversible changes were observed. This suggested that intra-arterial injection of HN_2 could produce, within the area supplied by the artery, an intense reaction from which normal tissue could recover. It also suggested a new method for treating regional carcinoma which has an accessible arterial blood supply.

The literature contains no reference to the intra-arterial injection of HN_2. However, the technic has been employed successfully for the injection of antibiotics,[8] heparin,[7] and vasodilators.[13]

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§ Hereafter referred to as HN_2

Intravenous HN_2 exerts some selective action against certain types of cancer in vitro[4] as well as in vivo.[16, 22] Some cases of carcinoma of the lung show a favorable response to intravenous HN_2.[3, 22] Histologic studies of tissues from these cases following HN_2 therapy have demonstrated cellular changes not seen in cancers in other parts of the body. While it is not certain how much of the blood supply of lung cancer is derived from the pulmonary artery as compared to the bronchial artery, the results obtained suggest that the increased concentration of HN_2 received through the pulmonary artery may have been the determining factor in the response obtained. This is closely comparable to intra-arterial therapy, for HN_2 injected into the antecubital vein passes directly into the capillary bed of the lungs. Thus, a cancer of the lung receives a much higher concentration of intravenously administered HN_2 than does a cancer distal to the pulmonary capillary bed. Similarly, by intra-arterial administration, a high concentration of HN_2 could be delivered to tumors elsewhere in the body, and the diluting and detoxifying factors attendant upon intravenous injection could be avoided.

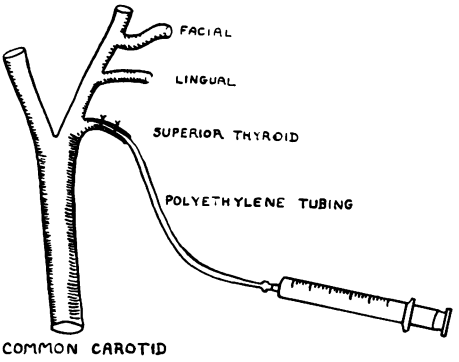


FIG. 1

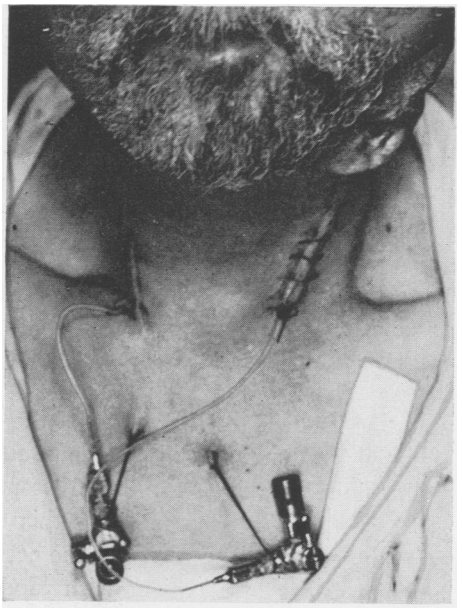


FIG. 2

FIG. 1.—Diagram illustrating the method of introducing the polyethylene tube into the external carotid artery.

FIG. 2.—Polyethylene tubes in the external carotid arteries, with the stopcocks attached and sutured to the skin of the chest (Case 1).

METHODS AND MATERIALS

A suitable technic was developed by utilizing the method which Donovan[7] devised for intraarterial administration of heparin. This method consisted of the introduction of a polyethylene tube through a proximal arterial branch directly into the artery selected for injection (Fig. 1). The tubing* used had

* Obtained from Anchor Plastics Company, New York, N. Y.

an inner diameter of .042 in. and an outer diameter of .068 in. The polyethylene tubes were prepared by inserting an intravenous needle of suitable size into one end of the tubing. Heating the needle caused the tube to contract firmly around the shaft. A stopcock was then soldered to the needle. In order to prevent the accidental release of the stopcock, all exit openings were closed with detachable connections which had been sealed with solder.

At operation the free end of the polyethylene tube was introduced into the proximal portion of the arterial branch as far as was desired. Silk ligatures were placed around the arterial branch over the tubing. Care was taken not to compress the tubing with the sutures or to injure it in bringing it through the incision. The protruding end of the tube was sutured to the skin to prevent accidental withdrawal (Fig. 2).

Before each injection, the patency of the tube was determined with the physiologic saline. The desired dosage of HN_2 was given into the tube, followed quickly by at least 5 cc. of physiologic saline solution. The nitrogen mustard used was the Methyl Bis (B-Chloroethyl) Amine Hydrochloride.* In each instance, the crystals were dissolved in physiologic saline solution (1.0 mg. per ml.).

ANIMAL EXPERIMENTS AND OBSERVATIONS

Experiments were set up to test the value of intra-arterial nitrogen mustard therapy for cancer.

Comparison between the effects of intravenous (total body) and intra-arterial (regional) HN_2 therapy on tissues of rabbits and dogs. In rabbits a single intravenous injection of HN_2 (1 mg. per Kg. of body weight) produced no gross changes in the extremities. However, a single intra-arterial injection of HN_2 (0.1 mg. per Kg. of body weight) into the femoral artery of each of six rabbits produced, three to five days later, demonstrable swelling and redness in the limb supplied by the injected artery. Within seven days after the injection, vesiculation and ulceration appeared over the distal portion of the extremity. This persisted for several weeks but subsequently healed.

Similar experiments were conducted on dogs. A single intravenous injection of HN_2 (0.1 mg. per Kg. of body weight) produced no gross changes in the skin, muscle, or extremities of the dog. A single intra-arterial injection of the same amount of HN_2 into the lingual artery of each of two dogs produced, three to six days later, marked edema in the portion of the tongue supplied by the injected artery. In 12 days, tissue destruction in the muscle and the epithelium was evident.

The effect of intra-arterial HN_2 on the Brown-Pearce tumor in rabbits. In order to test the degree of selectivity of HN_2 for neoplastic tissue in an animal in which the regional blood supply would be accessible for injection, the Brown-Pearce tumor in rabbits was selected. Brown-Pearce tumor was

* Supplied by Merck and Company as "Mechlorethamine Hydrochloride."

inoculated into the testes of 36 rabbits. When the tumors were large enough to be palpated, a single injection of HN_2 (0.1 mg. to 0.2 mg. per Kg. of body weight) was given into the abdominal aorta just above the orifices of the renal and spermatic arteries. The aorta was compressed distal to these vessels during the injection. Seven to ten days later the animals were killed and examined. Necrosis was present in all the treated tumors. The kidneys showed no histologic damage.

The effects of repeated intra-arterial injections of HN_2 on non-neoplastic tissues. Polyethylene tubes were inserted into the lingual arteries of normal dogs. One dog received five injections of HN_2 (0.2 mg. per Kg. of body weight) into the lingual artery at 24-hour intervals. Ulceration and necrosis of the tongue appeared on the tenth day. This destructive process was sharply limited to the ipsolateral tongue when the animal was killed and autopsied on the fourteenth day. Histologically, the lingual epithelium on the injected side was necrotic to the midline. At that point, there was a distinct line of demarcation between necrotic and normal epithelium. The demarcating line extended throughout the thickness of the tongue as a dark blue zone of necrotic and inflammatory tissue. On the untreated side, the tissue was normal except for hyperemia of the subepithelial vessels. On the injected side, all the tissues were necrotic, except for the fat, which had not been altered. The arterioles contained organizing thromboses, but the large arteries showed no change.

A second dog received six injections of HN_2 (0.1 mg. per Kg. of body weight) into the lingual artery at 24-hour intervals. The dog was killed and autopsied at the end of 14 days. The only pathologic finding was intense hyperemia of the ipsolateral tongue. Microscopically the lingual epithelium was intact on both sides. On the treated side, the underlying connective tissue showed moderate hyalinization. The muscle cells were shrunken and pale; and their nuclei were large, hyperchromatic, and irregular. Edema and small scattered areas of hemorrhage were present. Foci of inflammatory cells were scattered through the fatty tissue. The large arteries were normal, but the intima of the arterioles was thickened, and thromboses were present.

A polyethylene cannula was introduced into the internal carotid artery of each of five dogs, and 1 mg. of HN_2 was given every 24 hours for four to ten days. The injections produced no apparent pain or immediate effect. Within 72 hours, each dog had developed an enophthalmos, palpebral narrowing, and a suggestion of facial paralysis on the treated side. No convulsions were noted. The dogs would not eat after seven days but otherwise seemed in good health. At autopsy, the treated cerebral and cerebellar hemispheres were smaller, the superficial vessels more dilated, and the convolutions more flattened.

On cut section, soft, dark red areas were noted in the region of the basal ganglia. There was no change in either the cortex or the white substance except for the dilatation of vessels on the injected side. The only microscopic change in the injected hemisphere was hemorrhagic necrosis of the entire head

of the caudate nucleus and of the hypothalamus near the substantia nigra and the putamen. There was no change in the ependyma. There was no evidence of extra-cranial lesions or damage.

A polyethylene tube was introduced into femoral artery in each of two dogs, and 1 mg. of HN_2 was injected every 24 hours. After 48 hours, the hair on the treated leg could be plucked easily in large amounts, and the leg muscles were weak, although good arterial pulsations were present. One animal died in four days of peritonitis resulting from ischemia of the bowel due to inadvertent trauma to the mesentery at the time of operation. The second dog was killed and autopsied in ten days. No gross changes were apparent in the treated leg of either dog.

DISCUSSION

There was no available method of measuring the amount of the reactive HN_2 absorbed by the tissues, but it was possible to determine the effects of HN_2 by histologic study of tissues. The findings in these experiments were convincing, and the results were distinct and reproducible. It was apparent that intra-arterial injection of HN_2 produced, within the areas supplied by the injected arteries, tissue changes which cannot be produced by intravenous injection of lethal amounts of the drug.

In determining the degree of selectivity of HN_2 for neoplastic tissue, it was recognized that the Brown-Pearce tumor was not entirely satisfactory because of the high incidence of spontaneous necrosis. Though many animals would be required for quantitative studies to determine dosage, the constant presence of necrosis in all treated tumors, in the absence of damage to treated kidneys, indicated that there was a favorable selective action on this tumor.

The tissue response to HN_2 has been stated to be similar to that of irradiation.[10, 15] A single massive intra-arterial injection of HN_2, like a single massive dose of roentgen ray, produces complete destruction of all the treated tissues except fat and large blood vessels. A smaller dose of roentgen ray destroys only certain types of tissue. Repeated small doses of roentgen ray show even greater selectivity.[5] Because of the similar results obtained by the two types of massive dose therapy, an attempt was made to reproduce the selectivity of fractionated irradiation by repeated intra-arterial injections of small doses of HN_2. The results indicated that, by this chemotherapeutic fractionation, it was possible to produce in normal tissue, changes which are similar to those produced by fractionated irradiation.[18] The results also suggested the possibility that a cancerocidal dose of HN_2 might be delivered to a tumor by regional administration without producing local ulceration or necrosis of adjacent normal tissue.

CLINICAL TRIALS

The preliminary evidence of selective action of HN_2 by intra-arterial administration and the establishment of "safe" fractionated dosage levels in rabbits and dogs provided the basis for further studies on cancer patients.

This report covers the immediate results obtained in the first ten patients treated. We have excluded three patients who died from complications of their cancer within the first 24 hours of treatment. Results are tabulated on Tables I, II, and III.

The cases fall into three groups according to the anatomical region in which the tumor was located. Seven patients had cancer of the head and

TABLE I.—*Summary of Intra-Arterial HN_2 Therapy.*

Case	Pt.	Diagnosis	Previous Therapy	HN Therapy Total mg.	mg. Kg.	Dose Schedule	No. Days	Artery Cannulized
1.	J.H.	Epidermoid Ca. of upper lip metastatic to submaxillary glands	Surgery and Roentgen ray	76.7	1.47	1.3 mg. q. 8 hrs.	11	Rt. external carotid via superior thyroid
2.	J.S.	Epidermoid Ca. of soft palate	Surgery	41.0	1.10	1 mg.q. 24 hrs. then q. 12 hrs.	22	External carotids via rt lingual branch and left superior thyroid
3	L.L.	Epidermoid Ca. antrum metastases to submaxillary glands	Surgery and Roentgen ray	39.0	0.53	1.5 mg. q. 12 hrs.	12	Left external carotid via superior thyroid
		(2nd course 4 days after 1st)		41.0	0.55	2.0 mg. q. 8 hrs.	7	Rt. ext. carotid via lingual br.
4.	S.V.	Epidermoid Ca. right antrum with extension to palate and orbit and ethmoids	None	57.0	0.90	1.5 mg. q. 12 hrs. then 2.0 mg. q. 8 hrs.	11	Right external carotid via superior thyroid
	C.G.	Epidermoid Ca. of mouth with metastases to submaxillary glands	None	15.0	0.23	5.0 mg. q. 12 hrs.	7	Left external carotid via superior thyroid
		(2nd course 15 days after 1st)		50.0	0.76	2.0 mg. q. 8 hrs.	9	Left superficial temporal.
6.	D.M.	Epidermoid Ca. of right antrum	None	20.0	0.36	1.0 mg. q. 8 hrs.	7	Right external carotid via lingual
7.	B.B.	Epidermoid Ca. of mouth with metastases to submaxillary glands	Surgery	16.0	0.20	1.0 mg. q. 8 hrs.	6	Left external carotid
		(2nd course 13 days after 1st)		10.0	0.12	.5 mg. q. 8 hrs.	7	Superficial temporal
8.	D.L.	Fibrosarcoma of right thigh with metastases to lung	Surgery and Roentgen ray	122.4	2.45	2.5 mg. q. 12 hrs. then 3.3 mg. q. 8 hrs.	15	Right iliac via deep epigastric
		(2nd course 6 days after 1st)		32.9	0.66	2.5 mg. q. 8 hrs.	6	Right iliac via deep epigastric
9.	S.W.	Ca. of breast with cerebral metastasis	None	13.5	0.25	0.5 mg. q. 8 hrs.	16	Left common carotid via superior thyroid
10.	G.H.	Glioblastoma multiforme	Surgery and Roentgen ray	21.0	0.31	0.5 mg. q. 8 hrs.	14	Left internal carotid via superior thyroid

neck region supplied primarily by the external carotid arteries. Two patients had brain tumors supplied primarily by the internal carotid arteries, and one had a sarcoma of the extremity supplied by the femoral artery. The following case reports are representative of each respective group.

Case 1.—(J. H.): *Squamous carcinoma metastatic to both sides of the neck.* This 60-year-old colored male first noted a small ulcer on the upper lip in December, 1948. At the time of examination, July, 1949, a large fungating lesion had replaced two-thirds of the upper lip, and there were bilateral enlarged submaxillary lymph nodes. A biopsy

TABLE II.—*Effects of HN_2 Therapy.*

Case No.	Pain	Gross Changes in Lesion	Parasympathomimetic Ch nges	Gross Radiomimetic Changes	Course
1.	Uncontrollable prior to therapy. No analgesics required 16 hours after treatment.	Softening of tumor with discharge of necrotic m terial followed by partial healing.	Marked salivation. Miosis.	Erythema of face up to eyes; swelling of tongue and salivary glands. Localized depilation of scalp.	Died 89 days following institution of therapy.
2.	Severe pain in neck and throat completely relieved in 48 hours.	Regression in size of lesion.	Moderate salivation. Weakne s.	Mucositis of pharynx and soft palate.	Died on 25th day from massive pulmonary embolism.
3.	Persistent prior to treatment. Complete relief in 96 hours. Developed painful area on palate during therapy.	Softening and flattening of lesion by 6th day. Discharge of necrotic material through sinus tract.	Moderate salivation. Weakness?	Mucositis soft palate. Facial erythema.	Died 33 days following institution of therapy. Cachexia and terminal pneumonia.
4.	Frontal headache diminished in 48 hours, disappeared in 72 hours.	Ulceration and sloughing followed by shrinkage of oral, nasal and orbital mass.	Marked salivation. Skin right side of face warm and dry.	Mucositis. Dysphagia, hyperemia and edema of face.	Died on 26th day. Aspiration pneumonia?
5.	No pain prior to first course of therapy. Subsequent pain relieved by second course.	Softening and shrinkage of lesion; especially of neck nodes.	Excessive salivation. Skin of face warm and dry.	Hyperemia side of face followed by pigmentation and scaling.	Died 38 days following institution of therapy. Cause of death?
6.	Mild pre-treatment pain disappeared.	Reduction of lesion to less than one-half of original size.	Increased salivation. Skin right face warm and dry.	Small area of mucositis of hard palate. Local erythema.	Living 40 days after institution of therapy. Condition improved.
7.	Pain disappeared in 48 hours.	Ragged, bleeding tumor became smooth; bleeding stopped; reduced to ¼ of original thickness.	Excessive salivation.	Erythema over lower face. Mild mucositis.	Living 28 days after institution of therapy. Condition improved.
8.	None	Tumor became soft and cystic.	Skin of treated extremi y warm and dry. Increased weakness of foot questionable.	Erythema followed by vesicle formation. Depilation.	Died 29 days after institution of therapy. Cachexia.
9.	None	Could not be observed. Visual fields increased in size.	?Transient hemiplegia.	None.	Living 68 days after institution of therapy. Condition satisfactory.
10.	None	?Reduction in size and tension of subtemporal decompression.	None.	None.	Living 23 days after institution of therapy. Condition fair.

from the upper lip showed squamous carcinoma. On August 12, resection of the upper lip and bilateral upper neck dissections were performed. Histologic examination showed squamous carcinoma of the upper lip with metastasis to the submaxillary nodes bilaterally. For 3 months the patient was clinically free of disease. Recurrence was then noted both at the angle of the left mandible and in the right submaxillary region.

Roentgen ray therapy was started, but because the patient was unco-operative it was discontinued after he had received only 400 R. to each side of the neck. The tumor continued to grow rapidly and by November 25 he could swallow liquids only. Roentgenograms of the mandible demonstrated a large area of destruction in the posterior third of the horizontal ramus of the left mandible. Marked trismus limited the bite to a width of 2 cm.

TABLE III.—*Hematologic Effects of HN_2 Therapy.*

Case No.	W.B.C. Control	W.B.C. Lowest	W.B.C. Day	R.B.C. Control	R.B.C. Lowest	R.B.C. Day	Platelets Control	Platelets Lowest	Platelets Day	Bone Marrow Maximum Depression Day	Bone Marrow Maximum Depression Cellularity	Bone Marrow Maximum Depression % Maturing MN	E.S.R. mm./hour Before Therapy	E.S.R. mm./hour After Therapy
1.	12,500	1,200	13	4.41	3.33	6	315,240	53,100	22	10	++	4	40	28
2.	6,200	No fall		3.84	No fall		130,120	47,400	24	20	+++	56	11	30
3.	9,100	5,000	13	4.17	3.74	6	555,900	185,000	5	12	++	17	30	..
	*9,250	1,250	10	4.72	4.20	6	325,500	125,000	6	8	+	60	..	..
4.	5,600	800	20	4.22	3.78	16	144,520	64,530	19	14	+	74	21	23
5.	5,650	5,600	14	4.31	3.52	6	160,090	91,520	6	31	++	20.8	36	37
	*7,600	450	13	3.91	3.48	12	137,720	59,630	12	8	++	78.6	..	31
6.	5,700	6,300	8	4.28	3.96	8	175,480	153,500	6	No depression			32	34
7.	14,350	12,400	12	4.08	3.30	16	248,900	131,100	2	No depression			30	34
	*17,650	10,450	9	3.73	3.55	2	160,800	248,500	4	No depression			34	32
8.	13,700	2,950	18	3.97	3.05	14	177,100	42,540	10	13	++	51	34	32
	*3,950	1, 50	8	4.04	3.35	5	64,500	41,500	2	7	+	10	26	..
9.	7,300	6,450	12	4.35	3.72	5	243,900	179,000	14	No depression			41	48
10.	6,450	7,800	7	5.83	4.41	10	245,000	113,070	10	No depression			9	34

* Second course of therapy.

On November 27 the patient was admitted to The George Washington University Hospital. During the preoperative period he walked the floor and cried with intractable pain. On December 2, under general intratracheal anesthesia, a vertical incision was made along the anterior border of the right sternocleidomastoid muscle which was divided transversely. The external carotid artery was exposed, and its superior thyroid branch isolated and divided 2.5 cm. from its origin. A polyethylene tube was threaded, through the proximal portion of the thyroid tributary, into the external carotid artery and held in place by 3 silk ligatures. The tube was secured to the skin with a stitch ligature and the wound was closed with silk. The same procedure was carried out on the left side (Fig. 2).

Beginning 6 hours after the operation, 1.3 mg. of HN_2 (.025 mg. per Kg. of body weight) was injected into each polyethylene tube at intervals of 8 hours. This routine was continued for 10 days on the right side and 11 days on the left side for a total dose of 76.7 mg. Following each injection the patient experienced no pain, but occasionally complained of discomfort in the scalp. This was relieved by injecting 1 to 3 cc. of 1 per cent procaine into the tube. The right tube became plugged and was withdrawn on the tenth day without evidence of arterial bleeding. The left tube remained patent for 25 days.

Daily injections of physiologic saline were made after the eleventh day. An elective tracheostomy was performed on the eleventh day.

Serial biopsies obtained from the tumor in the left neck, every 2 days for 14 days and weekly thereafter, were studied microscopically to evaluate the effects of treatment on the tumor.

SUMMARY OF THE EFFECTS OF TREATMENT IN CASE 1

Effect on Pain. Within 16 hours after the first injection there was complete relief of pain and the patient no longer required opiates. Codeine was given for an occasional "headache."

Changes in Gross Appearance of the Cancer. (Figs. 3-6.) Before therapy, each ulceration on the neck had a firm, ragged, grey center and a hard, elevated, rolled edge. By the fifth day of treatment, each area of ulceration had enlarged, the rolled edge had disappeared, the tumor had softened, and the discharge was copious. By the ninth day, a sinus tract which extended into the left mandible was noted. By the eleventh day, both areas of ulceration had decreased in size. By the twenty-eighth day, healing was almost complete on the right side. However, on the left side residual carcinoma was present on the lower medial portion of the defect. This was the portion of the tumor supplied by branches of the superior thyroid artery which had been divided in cannulating the external carotid artery. No other active disease was apparent at this time. The sinus tract extending into the mandible remained.

Roentgenograms of the left mandible showed an increase in the area of destruction during the first 2 weeks of therapy, but no further increase in the next 8 weeks.

Gross Radiomimetic Changes. A slight reddish color appeared over both sides of the upper neck after three days of HN_2 therapy. The color was more intense after 5 days, and extended up to the lower eyelid on the left side to the malar bone on the right side. It persisted throughout the therapy and then slowly disappeared. Dysphagia developed on the fifteenth day, and tube feedings were required from the nineteenth to the twenty-fourth day. Painless trismus prevented adequate examination of the pharynx, but the dysphagia was attributed to edema. There was marked swelling over both parotid glands, and the tongue became tender and swollen after 8 days of therapy. The pressure of the swollen tongue against the 2 remaining lower teeth resulted in areas of necrosis which healed but left clean notches. The patient's beard ceased to grow, coincidental with the appearance of the erythema, and the hair of the scalp began to fall out one week later. By the twenty-fourth day, all the hair on the face and scalp was lost except for an inch-wide strip of sparse hair in the midline of the chin, lips, scalp, and the back of the neck. Even this had disappeared by the thirtieth day (Fig. 7).

Parasympatho-mimetic Changes. Increased salivation appeared on the eighth day and persisted for a month. Being unable to swallow, the patient drooled copiously. Atropine had no effect on reducing the amount of saliva.

Examinations of the eye were made periodically and no changes were observed in the fundi or cornea during or after therapy. One per cent Paredrine was effective in dilating the pupils until the twenty-sixth day, when it became necessary to use a more potent mydriate (10 per cent Neosynephrine).

Hematologic Changes. The hemoglobin and red cell count showed a moderate decline and then stabilized. This was coincidental with an increase in plasma volume which was attributed to increased fluid intake following relief of pain.

Two weeks after the initial injection, a mild macrocytic hypochromic anemia developed. The reticulocyte count remained normal. The platelet count began to fall by the sixth day, but remained above 100,000 until the twenty-fourth day, when it declined to 53,000. It remained low for 3 days, after which it returned to normal levels. The circulating lymphocytes were decreased in number within the first 24 hours. The few eosinophils noted in the first blood smears had completely disappeared by the third day. The total peripheral white count fell to 1200 on the fifteenth day and remained low for the next 11 days. The sedimentation rate was decreased during therapy and the serum protein levels diminished slightly. Calculation of total circulating protein indicated a slight increase in the albumin fraction and a decrease in the globulin fraction.

Bone marrow specimens were obtained twice each week. Eosinophilic leukocytes decreased markedly within one week. On the eleventh day, cellularity decreased from an

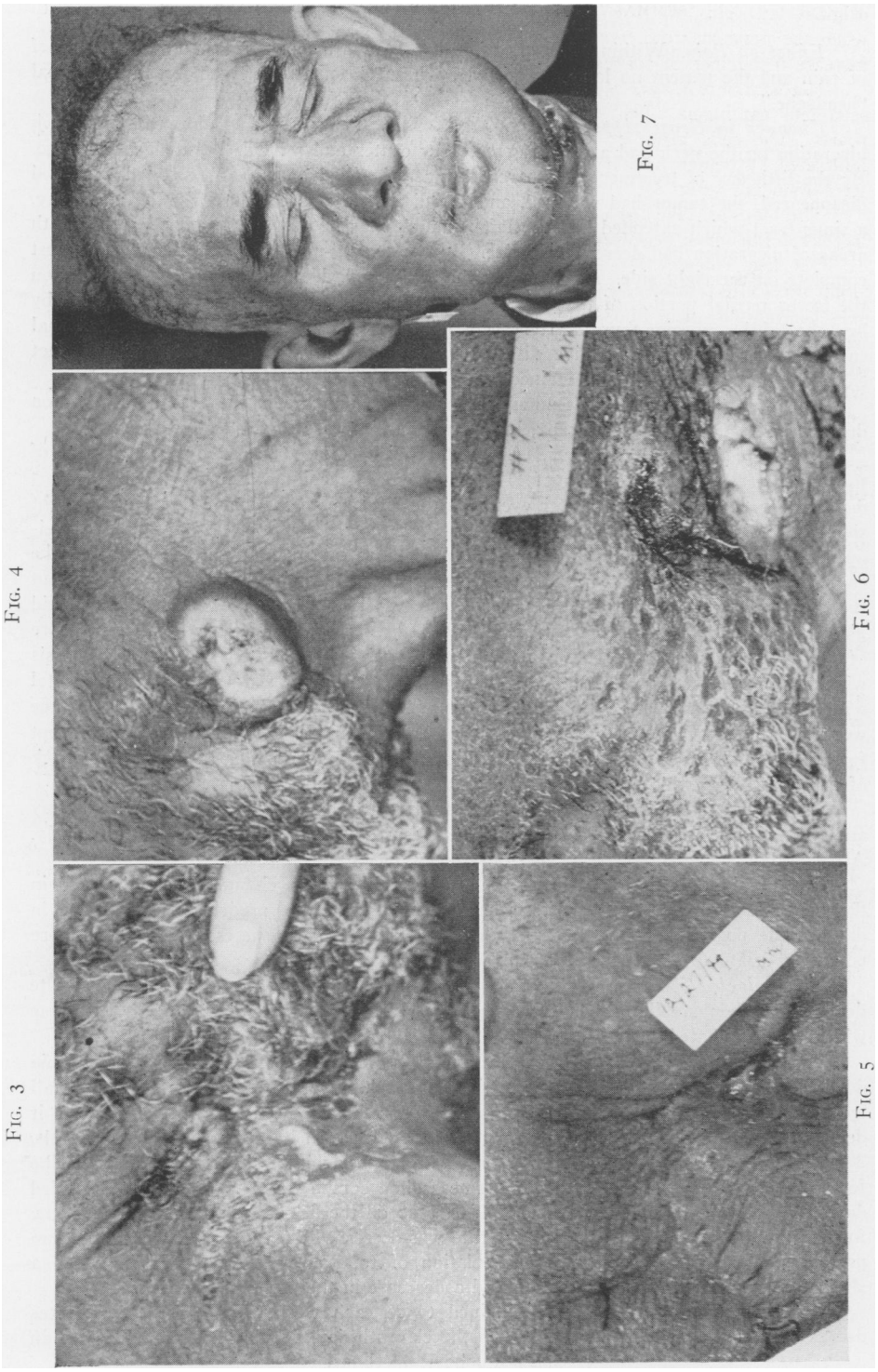


FIG. 3

FIG. 4

FIG. 5

FIG. 6

FIG. 7

(Legends on opposite page)

original four plus, to two plus. This persisted until two weeks after therapy ended, when the bone marrow first showed evidence of return toward normal as well as an increase in number of eosinophils.

Microscopic Changes in the Cancer. The pre-treatment biopsies showed a grade II squamous carcinoma. The tumor showed a tendency to keratinize and form epithelial pearls. The cells grew in large sheets, interspersed with keratinized masses heavily infiltrated by neutrophils, lymphocytes, and a few plasma cells. Normal mitotic figures were abundant (Fig. 8).

The biopsies, taken at intervals of 48 hours, all contained carcinoma but showed an increased amount of degeneration in successive specimens (Figs. 9, 10).

The first changes observed occurred after two days of treatment and consisted of slight intracellular edema, slight clumping of the nuclear material, and dilatation of blood vessels. In subsequent biopsies, intracellular edema increased and cell cytoplasm became pale pink and cell outline indistinct. Many small cytoplasmic vacuoles appeared and gradually enlarged throughout treatment. Neutrophils appeared in some of the vacuoles which almost filled the cells, compressing the nuclei into demilunes. A few of the enlarged cells, however, were well delineated by condensation of the peripheral cytoplasm. After ten days of treatment, the nuclei gradually enlarged, becoming more hyperchromatic with complete loss of detail and finally karyorrhexis occurred, and complete degeneration of the cells.

The total number of cancer cells per field was reduced approximately one-half after 6 days of treatment. The total number of mitoses per field was reduced by more than one-half after 4 days of treatment and most were bizarre forms. No mitotic figures were seen after 8 days of treatment and, after 12 days, the serial biopsies contained very few cancer cells, the tissue being composed of loose strands of edematous fibrous tissue, keratin and debris. Those cancer cells present were pale-staining epithelial cells which contained blue-grey homogenous karyorrhelic masses of nuclear material. Increasing amount of keratin and debris appeared in the biopsies throughout treatment, associated with an infiltration of lymphocytes and neutrophils.

Following 2 days of treatment, edema of the endothelial cells was the only vascular change. After 4 days of treatment, this appeared more marked and the intima of the arterioles became hyalinized.

At this time, a distinct halo of edema developed about these vessels. Subsequently, thrombosis occurred in most of the medium-sized arteries, followed by infiltration of lymphocytes and neutrophils. The larger arteries did not appear altered and the veins and capillaries showed only swelling of the endothelium.

The epithelium covering the treated area remained unchanged except for slight edema. Normal mitoses were present in the basal layer of the skin in all post-treatment biopsies. After the fourth day, the dermis showed progressive edema coincident with a lymphocytic infiltration. The fibrocytes became edematous and their nuclei hyperchromatic. These changes were more marked about the dermal arterioles.

CLINICAL COURSE

The first six injections of HN_2 caused nausea and vomiting. An unexplained temperature of 102° began on the twelfth day and continued at this level for the next six

FIG. 3.—Ulcerating, metastatic squamous carcinoma of right side of neck, before treatment (Case 1).

FIG. 4.—Ulcerating, metastatic squamous carcinoma of left side of neck, before treatment (Case 1).

FIG. 5.—Right side of neck 25 days after the beginning of therapy, showing almost complete healing of the ulcer (Case 1).

FIG. 6.—Left side of neck 21 days after beginning of therapy, showing partial regression of the portion of the ulcer supplied by the injected artery (Case 1).

FIG. 7.—Loss of hair following injections of HN_2 into both external carotid arteries (Case 1).

days. The patient was discharged from the hospital on the forty-sixth day. Roentgen ray therapy was later administered to the recurrent cancer in the left anterior neck, but he refused to co-operate and treatment was discontinued after 600 R. had been given. After this, he received 10, 10, and 5 mg. of HN_2 intravenously over a ten day period. Following this, the mass decreased in size, but the gross changes were not comparable to those which had been seen following the intra-arterial injection of the same amount of HN_2 He developed dysphagia, became weaker and was re-admitted to the hospital on February 21, where he died 4 days later. He was free of pain to the time of death.

FIG. 8

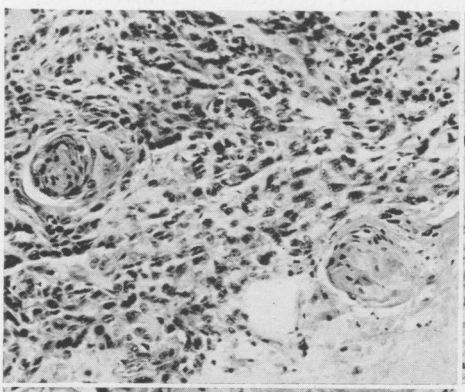
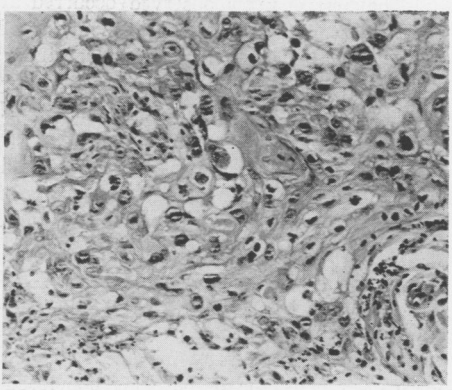


FIG. 9



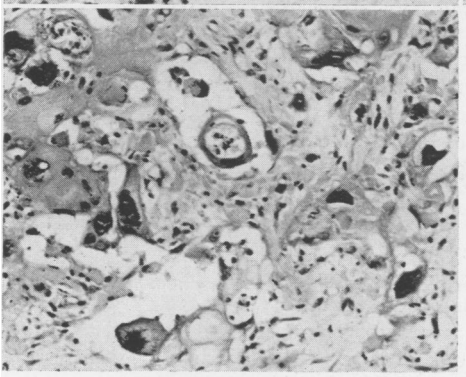


FIG. 8.—Pre-treatment biopsy of squamous carcinoma, Grade II, with tendency to keratinize and form epithelial pearls and intracellular bridges x 160 (Case 1).

FIG. 9.—Biopsy after four days of treatment, showing marked intracellular edema and cytoplasmic vacuolization with nuclear hyperchromatism. x 160 (Case 1).

FIG. 10

FIG. 10.—Biopsy after 12 days of therapy. The few remaining cancer cells are represented by large, irregular nuclear masses within thick-walled cells. These are embedded in a mass of degenerating keratin material and debris. x 160 (Case 1).

At autopsy, no tumor was found in the right side of the neck or in the left posterior region of the neck, either grossly or microscopically. Cancer was present in the untreated left anterior portion of the neck. Death was attributed to malnutrition and terminal pneumonia.

Case 2.—(S. W.) *Carcinoma, Metastatic to the Left Temporal Lobe.* This 30-year-old colored female was admitted on January 9, 1950. Her history was considered unreliable. Four months prior to admission she had discovered a lump in the left breast. One month prior to admission, she had noted a "glare" in the right eye and a decrease in

visual activity in both eyes and had suffered a convulsion, followed by a long period of coma. A clinical diagnosis of cancer of the breast with metastasis to axilla and brain was made. She was considered unsuitable for craniotomy.

On physical examination, she was drowsy. There was, in her left breast, a 9 by 5 by 5 cm. mass, in the upper outer quadrant, attached to the skin over a 4 cm. area. There were multiple hard axillary nodes in the left axilla. Aspiration biopsy showed carcinoma.

Her vision was 20/50. There was a paralysis of the right sixth cranial nerve. The left pupil was smaller than the right. There was no papilledema, no atrophy of the discs, but some muscular degeneration. There was a marked visual field defect (Figs. 11, 12).

Neurologic examinations were done by Dr. J. M. Williams. On admission, the patient was oriented as to the time and place. Spinal tap revealed an initial pressure of 380 mm. of water. No other abnormalities were noted.

On January 13, polyethylene tubing was inserted through the superior thyroid artery into the common carotid, and the external carotid artery was ligated beyond the orifice of the superior thyroid artery.

HN_2 (0.5 mg.) was injected every 12 hours for a total of 13.5 mg. Crysticillin (300,000 units) was given daily.

Visual fields enlarged (a malingering target was used) and the initial 15° limitation increased to a maximum limitation of 50° in 12 days (Figs. 13, 14). At this time unaccountable personality changes appeared and made the determination of further visual fields unreliable, so therapy was discontinued. The spinal fluid pressure was recorded at 170 mm. on the eighth day and at 280 mm. on the fifteenth day. Although unable to read during the period of hospitalization, she became able to read newspaper print one month later; the sixth cranial nerve paralysis had become less apparent.

The injection of HN_2 produced a mild transient burning sensation along the course of the internal carotid artery. On the sixth evening, a temporary lethargy appeared immediately after the injection. On the seventh day, sensory aphasia occurred after the injection and persisted for 48 hours. She gave an identical answer to all questions. However, she seemed to know the correct answer without being able to say it. On the ninth evening, a transient right hemiparesis appeared immediately after the injection. Motor control returned in the leg within 5 minutes, in the face within 10 minutes, and in the right arm within 15 minutes. A transient hemiparesis occurred again on the eleventh day. On the fourteenth day, it occurred following an injection of Thorotrast. On the eighteenth day, the cervical sympathetic nerves were blocked with procaine and a single injection of HN_2 was given. The hemiparesis recurred and persisted for 10 minutes.

During the entire period of hospitalization, the patient was ambulatory, fed herself and was free of pain. The primary cancer increased in size during treatment.

Hematologic Changes. No leukopenia or depression of bone marrow occurred. Blood volume was unchanged. The erythrocyte sedimentation rate rose from 41 to 48.

Case 3.—(D. L.) *Fibrosarcoma of Right Thigh, with Metastasis to the Left Lung.* This 61-year-old white male discovered a painless "lemon-sized" mass on the medial aspect of his lower right thigh in December, 1948. Local excision and histologic examination showed it to be a fibrosarcoma. The patient refused radical surgery. Within 2 months a recurrent nodule developed, was excised, and was proved to be fibrosarcoma. Five months later another nodule developed which, by September, had enlarged until it occupied the entire medial aspect of the right thigh. Amputation was again refused, so roentgen ray therapy was given. This consisted of a tumor dose of 1600 R. The tumor continued to grow. In December, 1949, chest roentgenogram revealed a large solitary metastasis in the left lung field. The patient was admitted to The George Washington University Hospital in January, 1950.

On physical examination, he was emaciated and weak. A large solid tumor mass occupied the entire postero-medial aspect of the right thigh. The circumference of the right thigh was three times that of the left. The skin over the tumor was taut and ulceration appeared imminent. Numerous patent dilated veins were visible beneath the skin.

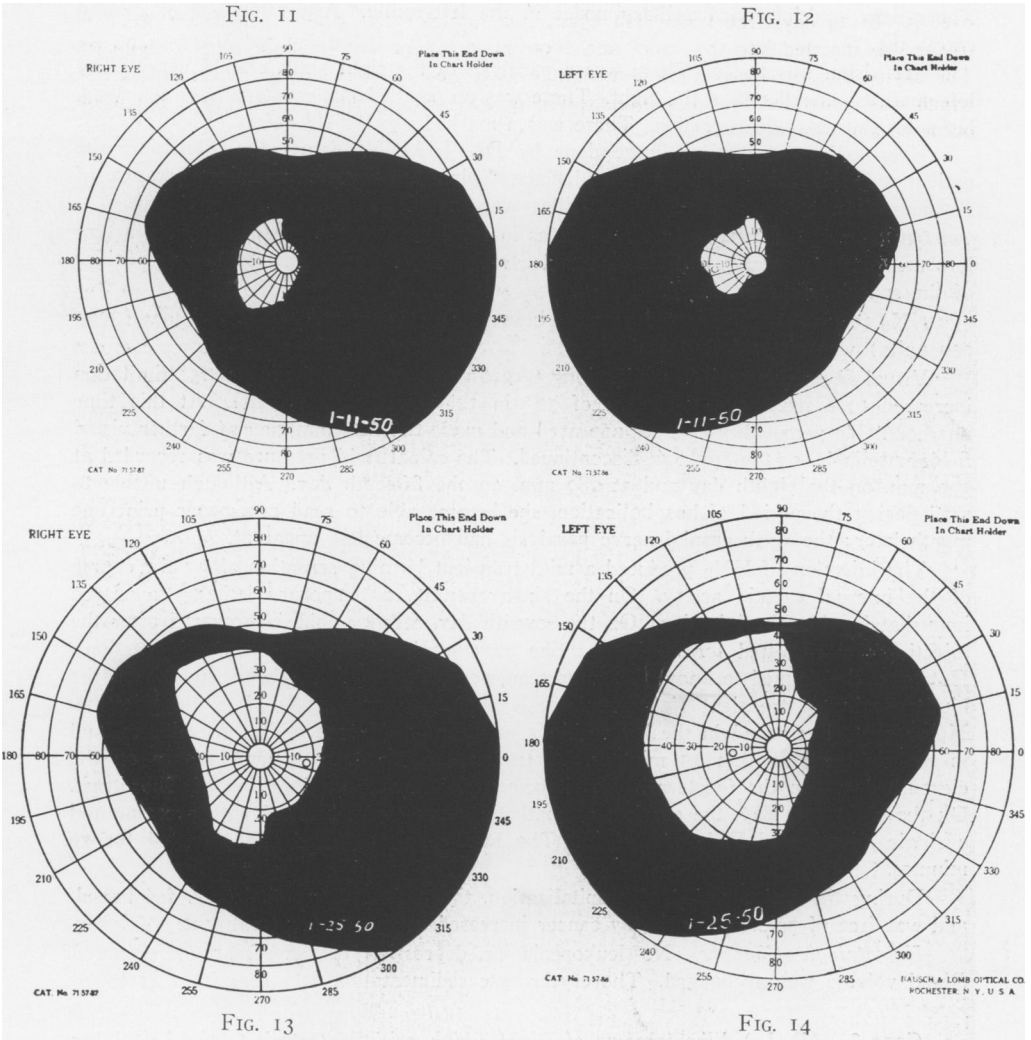


FIG. 11 FIG. 12

FIG. 13 FIG. 14

FIG. 11.—Visual fields before treatment (Case 2); right eye.
FIG. 12.—Visual fields before treatment (Case 2); left eye.
FIG. 13.—Visual fields after treatment by injection of HN_2 into the left internal carotid artery (Case 2); right eye.
FIG. 14.—Visual fields after treatment by injection of HN_2 into the left internal carotid artery (Case 2); left eye.

Marked pitting edema was present in the lower leg and foot. The weight of the left leg prevented ambulation, but there was no pain.

On January 27, a polyethylene tube was inserted through the deep epigastric artery into the right iliac artery, by Dr. A. Horowitz. Injections of HN_2 (2.5 mg.) were given

every 12 hours for 4 days, then increased to 3.3 mg. every 8 hours for a total dose of 122.4 mg. One week later, a second course was given consisting of 2.5 mg. every 8 hours for a total of 32.5 mg.

Effect on pain. The lesion was completely painless.

Gross changes in lesion. In 6 days, the tumor had softened and the skin was movable over it. In 14 days, the tumor felt cystic and a fluid wave could be demonstrated. A trocar was inserted into the tumor and 2300 ml. of viscous brown fluid were withdrawn. The cavity thus produced filled immediately with blood, and the patient went into shock which was controlled by the administration of 2500 ml. of blood. Thereafter, ulceration occurred and drainage was profuse.

FIG. 15

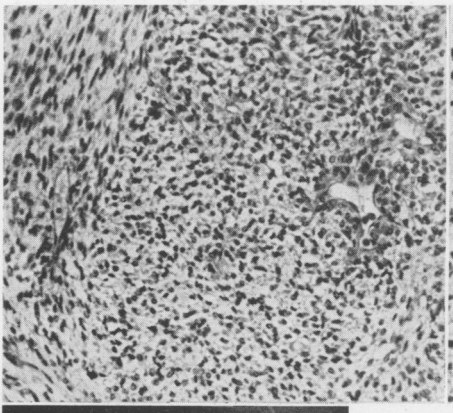
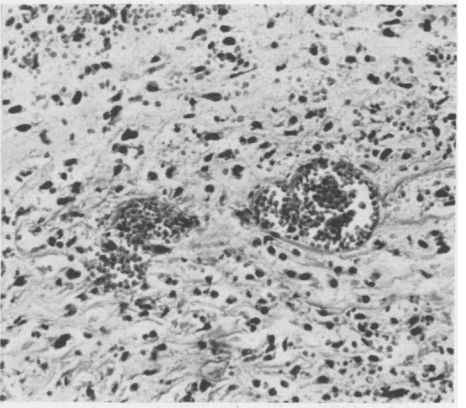


FIG. 16



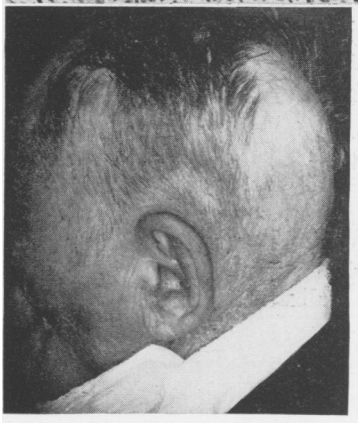


FIG. 17

FIG. 15.—Spindle call sarcoma of the right thigh, before intra-arterial HN_2 therapy. x 175, (Case 3).

FIG. 16.—Spindle cell sarcoma, following intra-arterial HN_2 therapy. x 175, (Case 3).

FIG. 17.—Alopecia limited to the area supplied by the occipital artery. The superficial temporal branch had been compressed during injection of HN_2 into the left external carotid (Case 3).

The lung metastasis increased in size during treatment.

Parasympathomimetic Changes. After the first few injections, the treated leg became warm and dry and there was a questionable increase in weakness of the muscles of the lower leg.

Radiomimetic Changes. After 6 days of therapy, hair could be removed easily from the calf but never fell out spontaneously. After the first course of therapy, a few small vesicles appeared on the thigh and lower leg. During the second course of therapy, an erythema developed over the thigh.

General Clinical Course. The HN_2 injections produced no nausea, vomiting, or pain. A low grade fever began during the second week of therapy and subsided immediately after the last injection of HN_2. Diarrhea developed on 2 occasions but was controlled easily with paregoric. During the 4 days before death, the blood pressure was 70/50.

The patient became progressively weaker, the skin was extremely pale; fecal and urinary incontinence developed. Just before death he became comatose and the temperature rose to 104° F. The last injection was given 5 days prior to death.

Microscopic Changes in the Tumor. The original tumor consisted of small, closely packed, spindle-shaped cells with indistinct cell membranes. The cells contained a small amount of clear cytoplasm and large deep staining, coarsely granular nuclei. Mitoses were frequent. There was no necrosis and the blood vessels were patent. The diagnosis was spindle cell sarcoma (Fig. 15).

At autopsy, the tumor consisted of a 4 cm. shell surrounding a multiloculated cavity. Only the lower pole of the tumor showed no central necrosis. The solitary lung metastasis was solid throughout. Microscopic sections of the tumor, obtained at autopsy 22 days after the first injection, showed large areas of necrosis, much debris, and had little resemblance to the untreated tumor (Fig. 16). Some cells were spindle-shaped, but others were irregular. The cytoplasm was distinctly outlined and deep pink, and the nuclei were irregular and extremely hyperchromatic. The cells were widely separated by edema fluid and contained scattered leukocytes. Surrounding the islands of residual tumor, there were many ghost cells and some hemorrhage. The large blood vessels were dilated, and their endothelium was edematous. A few small arterioles were thrombosed.

RESULTS

Complete and permanent relief of local pain was obtained in each case, whether the pain was produced by intra-oral ulceration, or by invasion of bone. The period of time between the first injection and the relief of pain was not related to the dose given, but was consistently less than 48 hours. A sense of well being was noted within two days and persisted until radiomimetic and parasympathicomimetic symptoms appeared.

All accessible treated tumors showed gross changes during treatment. The borders of ulcerated squamous cancers became soft and flattened, while their central portions liquefied. Discharge was copious and surrounding induration diminished. After two weeks of treatment, increased vascularity and a decrease in size were apparent. In three cases, epithelialization of the ulcer was almost complete.

The non-ulcerated treated squamous cancers responded more slowly but decreased in size and became softer. If subsequent ulceration occurred, initial discharge of liquefied material took place with a resultant immediate decrease in size.

Radiomimetic changes occurred in all except the brain cases, in which little surface area was included in the field of treatment. Skin erythema and local edema appeared within 14 days and were most marked in those cases in which larger individual doses were used. When oral mucous membrane was included in the field of therapy, whitish patches (similar to roentgen ray mucositis) appeared within ten days and persisted until after treatment was complete. When scalp or beard areas were included in the field of treatment, loss of hair

occurred within ten days. Eye lashes and eye brows were not affected. This loss of hair could be prevented by compressing the artery supplying an area of scalp, during each injection of the HN_2 (Fig. 17). Loss of hair was delayed in areas bordering either on the midline or adjacent non-treated areas. When a parotid gland was included in the field of treatment, marked edema developed within seven days and persisted throughout treatment. The intensity of these radiomimetic changes appeared to be directly proportional to total dosage and inversely proportional to the duration of treatment. Of cases receiving the same total dose, those treated over the longer period had less intense reactions. Of cases treated over the same period of time, those receiving the largest total dose had the most intense reactions.

Parasympathomimetic effects were present in each case in which there was, within the field of treatment, an end organ whose response to parasympathetic stimulation was measurable. Increased salivation always occurred when HN_2 was injected into the external carotid artery but was limited to the ipsolateral glands. In one case, in which a large total dose was given into the external carotid artery, a miosis developed which could not be completely counteracted by the usual mydriatics. Skin areas within the treated region became warm and dry during the period of treatment and for several days thereafter.

Certain neuromuscular disturbances were noted, but the mechanism concerned in their production was not clear. We have chosen to consider them under the general classification of parasympathicomimetic effects. Convulsions occurred in two cases, but were secondary to anoxia following pulmonary embolus in one. In the other case they occurred immediately after the injection into the internal carotid artery of HN_2 and of Thorotrast. The injection of HN_2 into the internal carotid artery did not produce convulsions in the other patient, who received even larger single doses. Five cases developed marked weakness during the second and third week of treatment. In three instances, this was followed by the appearance of asynchronous fibrillary muscle twitching in all extremities and by marked prostration. At the same time, the pulse was not rapid, the extremities were warm and dry, but the blood pressure was below normal. The patients were lethargic but could be aroused and were oriented. These symptoms persisted until death occurred, which was associated with bronchopneumonia in one case, with massive aspiration of vomitus in the second, but with no known cause in the third.

The blood studies have been summarized in Table III. A significant leukopenia developed in only four patients, reaching a maximum between the thirteenth and twentieth day of treatment. The eosinophils always disappeared from the peripheral blood within four days and reappeared ten days following completion of therapy. A lymphopenia always developed within four days and persisted until at least eight days following completion of therapy. The degree of lymphopenia appeared to be proportional to the amount of HN_2 which was estimated to have passed into the general circulation rather than to the

amount in each individual dose or to the total dose administered. Partial mechanical block to venous return from the primary treated area seemed to decrease the severity of the lymphopenia and leukopenia, whether by bilateral removal of the efferent veins or by application of a venous tourniquet proximal to the site of injection.

An initial polymorphonuclear leukocytosis always occurred and persisted up to nine days. Toxic granulations appeared in the neutrophiles and were present throughout therapy. Vacuolization of monocytes was striking during the recovery phase, and numerous unclassified plasma cells appeared.

Cellularity of bone marrow was estimated and recorded as one, two, three, or four plus (normal). A depression of bone marrow occurred in all cases which received more than a total of 20 mg. of HN_2. It reached a maximum of one to three plus in seven to 31 days. Multiple injections in small doses produced the least depression. At the time of maximum depression, the marrow showed a high percentage of adult polymorphonuclear neutrophiles.

The red blood cell count and hemoglobin were not affected by therapy even though there was considerable depression of normoblast in the bone marrow. These patients have not been followed long enough to detect a late anemia.

All patients showed some depression of platelets, but none developed petechiae or purpura. The course of the platelets was extremely erratic, and no general statements are warranted.

Histologic changes occurred in all treated tumors which could be examined. In the squamous cancers, the first changes were observed on the second day of treatment and consisted of extracellular and intracellular edema of all treated cells except those in the epidermis. Subsequent biopsies showed increasing degeneration of the cancer cells, loss of nuclear details, cytoplasmic vacuolization, and thickening of the cell membrane. Mitoses became reduced in number, and many were abnormal in appearance. Keratin and debris appeared in increasing amounts. Thromboses occurred in some arterioles, but the veins and large arteries showed only endothelial swelling. The stromal cells showed slight edema and minimal degeneration. The basal layers of the epidermis showed slight edema and normal mitoses throughout therapy.

Progressive degenerative changes could not be observed in the case of the fibrosarcoma, since serial biopsies could not be taken. Sections removed at autopsy 22 days after the first injection, however, showed marked destruction of the cells, leaving only scattered islands of recognizable sarcoma which was in the process of cytolysis.

DISCUSSION

The treatment of a malignant tumor by repeated injections of a chemotherapeutic agent into its nutrient artery is a method of cancer therapy not previously described. By this method, an increased amount of HN_2 has been delivered to certain malignant tumors, and measurable changes have been produced which have not been seen to follow the intravenous administration of

the same drug. Since we have undertaken these studies, we have learned of the work of Shimkin,[21] who has obtained similar results following the single intra-arterial injection of HN_2 in patients with skin manifestations of a malignant lymphoma.

The regions best adapted to this type of therapy will be determined by the anatomy of the arterial blood supply. A tumor, located in an area whose blood supply is derived mainly from one artery, would be most suitable. Therefore, tumors occurring on an extremity or in certain areas of brain, head, neck and pelvis could be treated. Those occurring in areas within the thorax, upper abdomen and either thoracic or abdominal wall would probably require quite complicated technics. However, a tumor within a specific organ such as liver, might be treated, but this would first require a study of the possible toxic effects of the drug on each organ in question.

Detailed knowledge of vascular anatomy is necessary, as the entire tumor area must be included. Failure to define accurately the treated areas would and has resulted in either rapid recurrence of the tumor in an under-treated border zone, or failure to obtain any regression of tumor located just beyond the region supplied by the artery through which the drug is injected. The sharpness of the boundaries of the treated region is illustrated in the case in which the area of epilation on the scalp was confined to that supplied by the occipital artery, other scalp arteries being compressed at the time of injection (Fig. 17).

The ideal agent for this type of therapy may not be HN_2, which was the only drug employed in these studies. The ideal drug would be extremely toxic to cancer cells, but quite innocuous to adjacent normal tissue cells. It would be either completely absorbed by the tissues adjacent to the first capillary bed, or completely inactivated by the time it reaches the venous circulation. Few drugs have been studied with a view to defining these properties.

Methods are available either to increase the intensity of the regional effect, or to decrease severity of systemic toxic effects. Application of a venous tourniquet proximal to or ligation of veins efferent from a treated area should increase both the circulation time through the first capillary bed, and the intracapillary pressure, and thus increase both the amount of the drug delivered to the tissues of the first capillary bed, and the time during which de-toxification could take place. These methods were used and it was our impression that they were effective. Intravenous injection of a non-toxic antagonist at the time of each intra-arterial injection of the chemotherapeutic agent should reduce the toxic systemic effects of the agent. Such use of sodium thiocyanate as an antagonist to HN_2 has been suggested[2] and has merit. The use of a heart-lung mechanical pump to maintain the circulation of an isolated tumor-bearing extremity during the period of therapy would prevent action of the drug on the remainder of the body.

All these methods should be evaluated. The presence of the tumor itself may have a protective effect. Adair and Bagg[1] treated a few malignant tumors

in human patients and animals by local application of mustard gas. They noted that the presence of a malignant tumor seemed to afford some degree of protection again large doses of mustard gas. It was also our impression that the patients whose treated regions contained the largest tumors had less systemic toxic effects from the HN_2. It is also important that this be determined accurately.

Neither the optimum total dosage nor rate of administration of HN_2 has been determined. Almost universally, total dosage only has been considered in studies of total body cancer chemotherapy. Considering the analogy that the effectiveness of radiation therapy has been increased by either repeated small doses (Coutard X-ray Therapy) or by continuous therapy at a lower intensity (interstitial radium therapy using low intensity needles), it would not be unreasonable to assume that a dosage schedule is of considerable importance in regional cancer chemotherapy. It has been our impression that regression was slower, but that toxic action on regional normal tissue was less, when the intervals between treatments were 12 to 24 hours, than when the intervals were eight hours. However, long intervals between injections increased the total time during which the arterial cannula had to remain patent, and thus increased the technical difficulties in delivering a maximum total dose of drug. It is important to determine the optimum intervals between injections and the advisability of using continuous slow, intra-arterial perfusion.

The possible advantages of this type of therapy are great. If complete chemical control of the local regional cancer were possible, some extensive operations could be avoided. If only partial control of the local cancer were possible by regional chemotherapy, supplementary roentgen ray therapy might be used to complete the treatment.

The synergistic action of HN_2 and roentgen rays has been demonstrated to apply to total body therapy; therefore, regional roentgen ray therapy as a supplement to regional chemotherapy should be equally effective. Serial biopsies of accessible treated cancers have demonstrated marked destruction of tumor cells and changes which are almost indistinguishable from those following treatment with cancercidal doses of roentgen or gamma rays.[11, 25] Observation of changes in the gross appearance of ulcerated cancers during treatment have yielded similar results, while skin changes in adjacent treatment areas have been minimal. Much can be learned by further studies of this "chemical radiation." There is a suggestion that malignant tumors which are insensitive to radiation may be sensitive to the effects of regional chemotherapy. This was demonstrated both in the case of the fibrosarcoma which had shown no apparent response to external irradiation, and in the case of the squamous cancer, recurrent after a full therapeutic course of local external irradiation. Both of these cases showed regression following regional HN_2 therapy.

Our initial results are encouraging. Local pain, due to tumor, was relieved at once and did not recur even in those patients who survived several months.

Complete autopsy studies are now available of the first two cases treated. In one case there was neither gross nor microscopic evidence of cancer within *the treated region.* There was untreated and metastatic cancer in other regions of the body.

Our experience has been limited and, until many of the problems mentioned have been solved, regional intra-arterial cancer chemotherapy must be considered as an experimental procedure. By injudicious use of this method, one could produce necrosis of all tissue within a treated region. No rules as to dosage or schedule of treatment can yet be formulated. Because of the encouraging initial clinical results, further studies should be undertaken in suitable institutions.

SUMMARY

1. A method of regional cancer chemotherapy is described.

2. The intra-arterial injection of HN_2 produces within the area supplied by the injected artery, a more intense tissue reaction than can be produced by the intravenous administration of lethal doses of the same drug.

3. HN_2 administered intra-arterially in repeated small amounts, has been shown to have a selective action on neoplastic tissue within the area supplied by the injected artery. Gross and microscopic evidence of regression has been demonstrated in clinical areas.

4. Animal and clinical studies are included in this preliminary report.

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