Carcinogenicity of Diazoacetic Ester Administered Intravenously to Rats (35023)

B. T. So,[1] H. Druckrey, S. Ivankovic, R. Preussmann
(Introduced by P. Shubik)

Forschergruppe Präventivmedizin am Max-Planck-Institut fur Immunbiologie in Freiburg i. Br., Germany

After the discovery by Magee and Barnes (1) in 1956 that dimethylnitrosamine induced liver tumors in rats, Druckrey and co-workers studied a series of nitrosamines (2) for the relationship between chemical structure and carcinogenicity. The site of origin of induced tumors appeared to be dependent not only on the chemical structure of the carcinogen, but also on factors such as dose and route of administration (3). It was of interest to test diazoacetic ester (DAAE), known to induce carcinomas of the forestomach when given orally (4), by iv administration.

Materials and Methods. DAAE was synthesized in our laboratory and checked for purity by UV spectral absorption. 90-day-old rats of BD IV and BD VIII strains were used. They were maintained on pelleted Altromin diet supplemented by carrots and salads twice weekly. Drinking water was given *ad libitum*. Rats were examined daily and weighed weekly until natural death or were killed when moribund. Complete autopsies were performed; tumors and pathological organs were studied microscopically.

Experiments. The acute LD_{50} by intravenous injection was 280 mg of DAAE/kg of body weight. After the injection of DAAE, the animals appeared apathetic, cyanotic, and dyspneic, dying shortly afterwards. Autopsy showed that the organs, especially liver, spleen, and blood were dark blue in color. For the chronic toxicity test, the rats received weekly intravenous injections of DAAE until thrombosis of the tail veins prevented further treatment. Animals were divided into four dosage groups as shown in Table I.

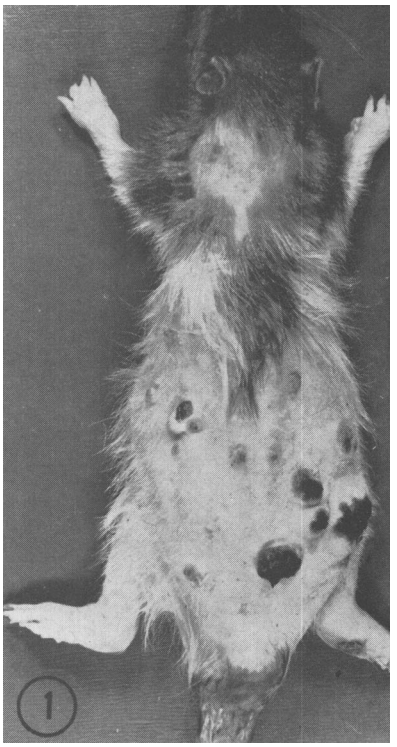

Fig. 1. Multiple skin tumors in a BD VIII rat treated iv with diazoacetic ester.

Results and Discussion. As shown in Table I, 13 out of 18 rats developed skin tumors (Figs. 1 and 2). This is a significant finding because the rat skin is very resistant to chemical carcinogens (5, 6) and because the DAAE was not directly applied to the skin. In 1963 Morris *et al.* (7) induced skin ap-

[1] Present address: Eppley Institute for Research in Cancer, University of Nebraska, College of Medicine, Omaha, Nebraska 68105.

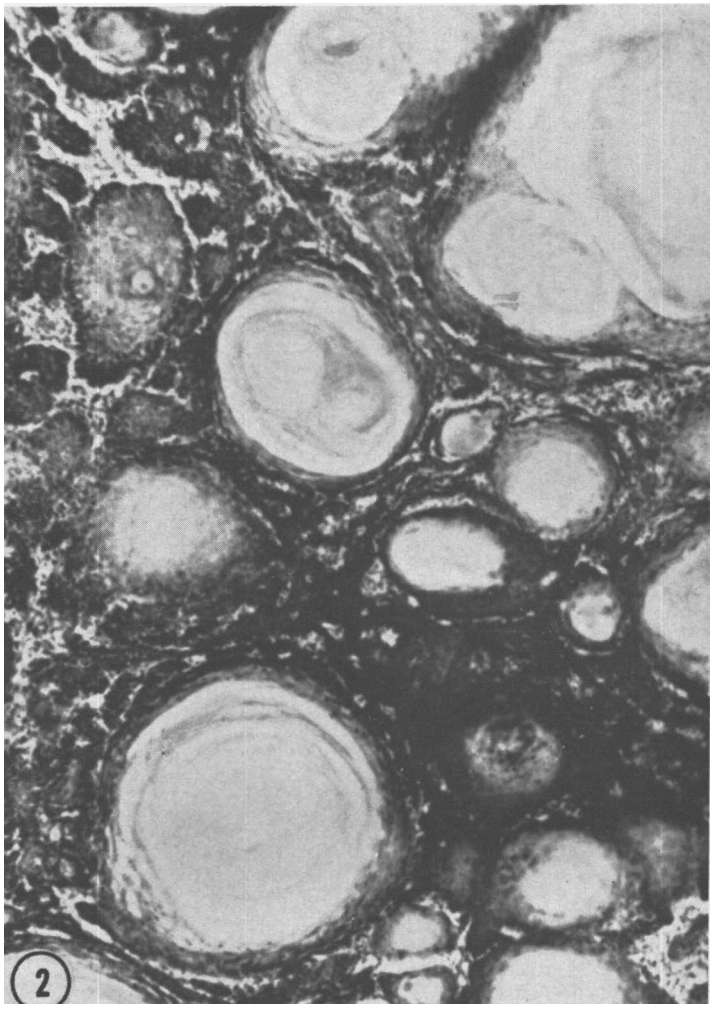

FIG. 2. Squamous cell carcinoma induced by diazoacetic ester in a BD VIII rat, after iv administration.

pendage tumors in rats after oral administration of *N,N'-2*, fluorenylenebis-2,2,2-trifluoracetamide. Of more than 65 *N*-nitroso-compounds tested by Druckrey and coworkers, DAAE is the only one that induced skin tumors when given intravenously (8, 9).

DAAE can be easily transformed to the very reactive diazonium ion at acid pH (4). When given orally, tumors in the stomach develop presumably because of the acid pH of the gastric cavity. Other tumors induced by DAAE are listed in Table I.

Three rats with skin tumors also presented lung tumors of the same histological type; whether these tumors are metastases or primary tumors is difficult to answer at the moment (Fig. 3).

Summary. Eighteen BD IV and BD VIII strain rats were treated intravenously with diazoacetic ester (DAAE). Six rats received 50 mg/kg of body weight and the rest 25 mg/kg of body weight. After total doses of 1000, 1600, and 2100 mg/kg of body weight, the treatment was stopped because of thrombosis of veins. After 400 days, 13 rats presented multiple skin tumors histologically

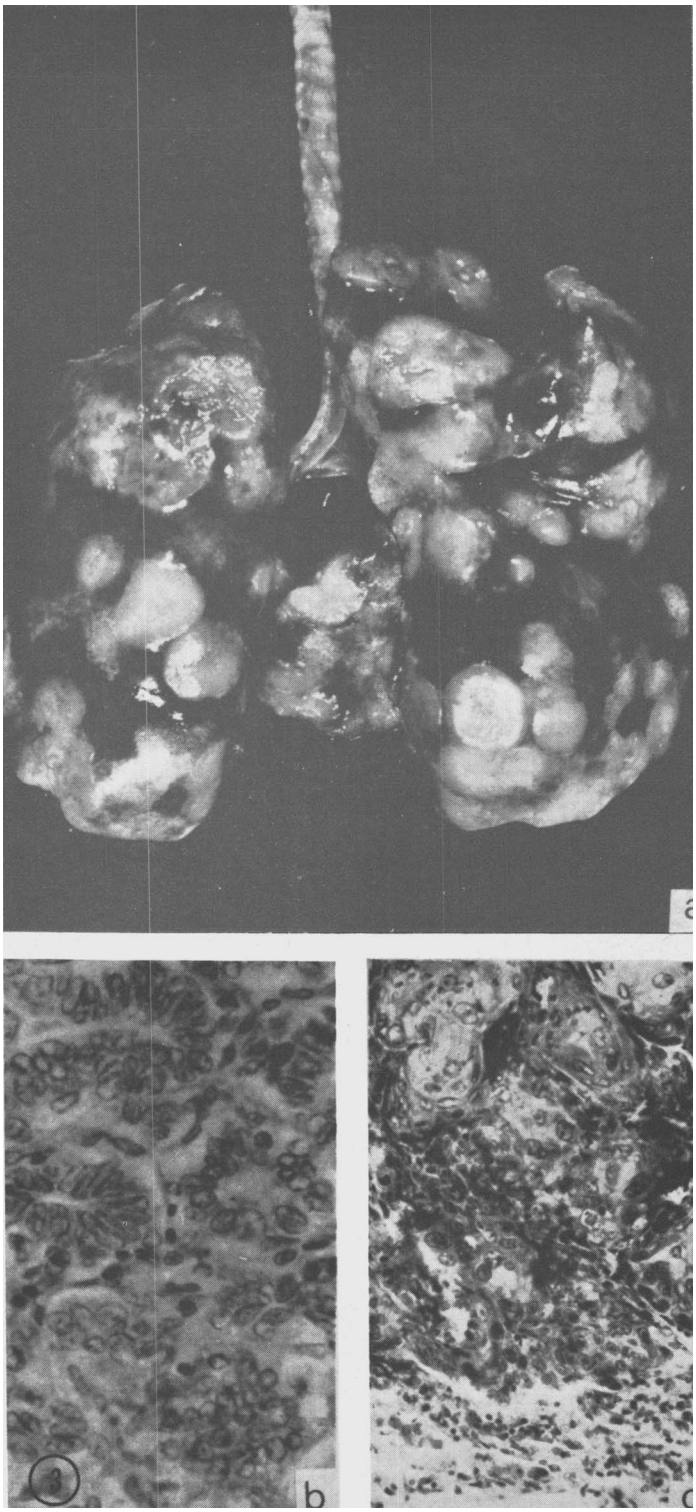

FIG. 3. Multiple lung tumors in BD IV rat induced by iv administration of diazoacetic ester: (a) macroscopic appearance; (b) adenocarcinoma; (c) squamous cell carcinoma.

TABLE I. Tumors in Rats Treated by Diazoacetic Ester iv.

Group	Animal no.	Dose[a] Weekly	Dose[a] Total	Survival (days)	Tumor Site	Tumor Type
1	1	50	1000	397	—	—
	2	50	1000	726	Skin	—[e]
					Mammary gland	—[e]
					Ovary	—[g]
	3	50	1000	783	Skin	—[bc]
2	1	50	2100	331	Mammary gland	—[e]
	2	50	2100	594	Skin	—[cd]
	3	50	2100	631	Skin	—[c]
3	1	25	1000	498	Skin	—[bc]
					Parotid gland	—[b]
	2	25	1000	565	Skin	—[bc]
					Lung	—[bcf]
	3	25	1000	607	Skin	—[b]
					Lung	—[b]
	4	25	1000	695	Mammary gland	—[e]
	5	25	1000	920	Pituitary	—[e]
	6	25	1000	812	Mammary gland	—[e]
					Pituitary	—[e]
4	1	25	1600	547	Skin	—[c]
	2	25	1600	587	Skin	—[bd]
					Lung	—[b]
	3	25	1600	587	Skin	—[c]
	4	25	1600	612	Skin	—[b]
	5	25	1600	615	Skin	—[bc]
	6	25	1600	647	Skin	—[d]

[a] (mg/kg of body wt).
[b] Squamous cell carcinoma.
[c] Basal cell carcinoma.
[d] Epithelioma sebaceum.
[e] Adenocarcinoma.
[f] Alveolar cell carcinoma.
[g] Papillary carcinoma.

diagnosed as basal cell carcinomas, sebaceous epitheliomas, papillomas, and squamous cell carcinomas. Of 13 rats with skin tumors, 3 also had lung tumors. Seventeen out of 18 rats developed some type of malignant tumor.

1. Magee, P. N., and Barnes, J. M., Brit. J. Cancer **10**, 114 (1956).

2. Druckrey, H., Schmähl, D., and Dischler, W., Z. Krebsforsch. **65**, 272 (1963).

3. Druckrey, H., Ivankovic, S., Mennel, H. D., and Preussmann, R., Z. Krebsforsch. **66**, 138 (1964).

4. Druckrey, H., Preussman, R., Blum, G., Ivankovic, S., and Afkham, J., Naturwissenschaften **50**, 100 (1963).

5. Takayama, S., Gann **53**, 167 (1962).

6. Nakahara, W., Arzneim.-Forsch. **14**, 842 (1964).

7. Morris, H. P., Wagner, B. P., Ray, F. E., Stewart, H., L., and Snell, K. G., J. Nat. Cancer Inst. **30**, 143 (1963).

8. Druckrey, H., Ivankovic, S., and So, B. T., Z. Krebsforsch. **66**, 523 (1965).

9. Druckrey, H., Preussmann, R., Ivankovic, S., and Schmähl, D., Z. Krebsforsch. **69**, 103 (1967).

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