Early Changes in Rat Adrenal Protein Synthesis After Dimethylnitrosamine Administration[1] (36683)

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(Introduced by P. K. Dixit)

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A single administration of dimethylnitrosamine (DMNA) produces centrolobular hepatic necrosis (1); prolonged feeding produces hepatic and renal neoplasms (2). The mechanism by which cell injury or cell transformation is brought about is not known. Evidence has now been presented that the action of the toxin is modulated by relative rates of metabolism, principally by the liver, and that this metabolism is further modified by adrenal hormone production. For example, adrenalectomy reduces the potential of dimethylnitrosamine hepatic carcinogenicity (3), and the acute rebound effect on protein synthesis and glycogen accumulation in the liver is related to adrenal function (4–6). For these reasons we undertook an analysis of protein synthesis in rat adrenal glands in response to a single dose of dimethylnitrosamine. We chose protein synthesis as an index of adrenal activity because we felt it would serve both as a monitor of cytoplasmic changes in response to the toxin, and reflect the adrenal response to the action of corticotropic hormone [see (7)].

Materials and Methods. Male Sprague-Dawley rats weighing between 160 and 220 g were fasted 16 to 19 hr, but were permitted water *ad libitum*, before use. Gastric intubation of DMNA (5 mg/ml water) was at a dosage of 20 mg/kg body weight. Intraperitoneal injection of Celite (45 mg/ml) or dimethylnitrosamine (5 mg/ml) in 0.9% NaCl was administered at doses of 225 or 20 mg/kg, respectively. Control rats received equivalent volumes of water or saline by the corresponding route. One to 24 hr after treatment, animals were anesthetized with ether, the abdomen was opened, and the viscera were perfused with cold saline via the thoracic aorta. The adrenal glands were removed, washed and homogenized in 3 vol of a 0.15 *M* sucrose solution containing 75 m*M* KCl, 10 m*M* $MgCl_2$, and 35 m*M* Tris buffer (pH 7.8) at 20° (8). The brei was centrifuged for 5 min at 15,000*g*. Aliquots of the resulting supernate were incubated for 15 min at 37° in a final volume of 100 μl containing 2 m*M* ATP, 10 m*M* PEP, and 0.147 m*M* L-leucine-1-^{14}C (30–34 mCi/mmole). Aliquots (25 μl) of the incubation mixture then were transferred to 2.4 cm filter paper disks (Whatman No. 540) and processed by the method of Mans and Novelli (9). The radioactivity of the disks was measured by liquid scintillation spectrophotometry in 5 ml of toluene containing 0.4% 2, 5-diphenyloxazole and 0.02% 1, 4-bis-2-(5-phenyloxazolyl) benzene. Counting efficiency for ^{14}C was 48%. Protein contents were estimated by the method of Lowry *et al.* (10).

Results. Intragastric administration of DMNA resulted in a prompt increase in amino acid incorporation (Fig. 1) with a return toward control levels by 8 hr. Detailed analysis of this response at 3 and 12 hr revealed a greater than 50% rise in the rate of leucine incorporation (Table I). The observed response might be the result of a direct adrenal effect of dimethylnitrosamine, or it might be the response of the adrenal axis to a stress associated with the drug administration. Direct addition of DMNA (1 m*M*) to adrenal slices incubated in Krebs–Ringer–bicarbonate buffer (11) for 1 and 2 hr revealed only a slight decrease (16%) in amino acid incorporation, whereas liver slices similarly treated

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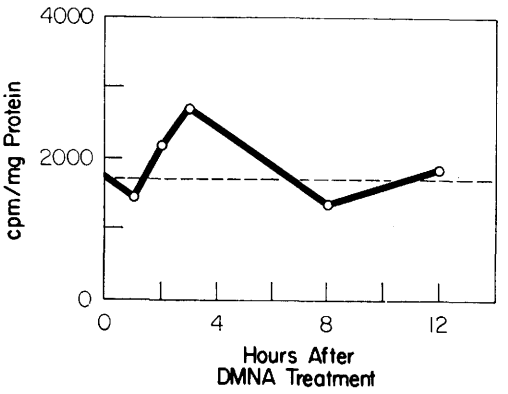

FIG. 1. Effect of gastric intubation of dimethylnitrosamine on *in vitro* L-leucine-1-^{14}C incorporation into rat adrenal protein. (- - -) The average of control values.

showed a marked inhibition of protein formation (12).

To test if the observed response was related to an adrenal response to the "stress" associated with DMNA effects to the animal, a series of experiments were carried out in which Celite (acid washed diatomaceous earth) was used to produce an adrenal mediated stress (cf. 13). Since this material must be given by intraperitoneal injection, parallel experiments were carried out with DMNA administered by the intraperitoneal route.

Intraperitoneal injection of DMNA resulted in a prompt augmentation of amino acid incorporation by adrenal preparations, reaching an early maximum around 2 hr, falling to near controls at 4 hr and then showing a second rise at 12 hr with a return toward control levels by 24 hr. This biphasic response was somewhat different from the prolonged and sustained stimulation of Celite (Fig. 2 and Table II).

Discussion. The evidence presented indicates that administration of DMNA results in enhanced amino acid incorporation by adrenal preparations. The biphasic nature of the change, when compared to Celite induced stress, suggests that the response is complex. The early stimulation occurs during peak concentrations of DMNA in blood; the difference in the early maximum effect in intragastric and intraperitoneal responses may represent differences in rates of absorption. The absence of a direct stimulatory effect on adrenal slices suggests that DMNA by itself may not be the active agent. Since DMNA is metabolized principally by the liver, it may be that its metabolities are responsible for the enhanced adrenal protein synthesis. The late accentuation of synthetic activity occurs after the DMNA has been removed and during a time of significant liver injury, suggesting that both phases may represent a stress rather than a direct effect of the toxin. Support for this comes from histologic examination of the response to the intraperitoneal administration of the agents. Celite injection is associated with an immediate and prolonged exudative response with significant

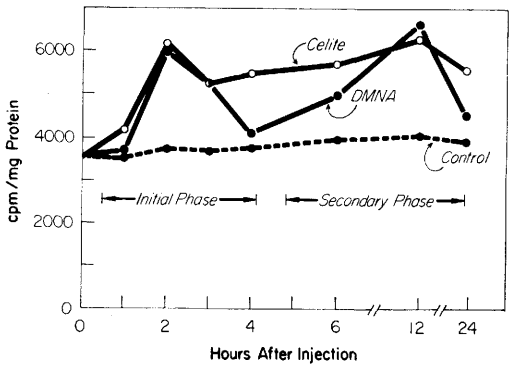

FIG. 2. Effects of intraperitoneal injections of Celite or dimethylnitrosamine on *in vitro* L-leucine-1-^{14}C incorporation into rat adrenal protein. Each point represents the average of 4 or more determinations.

TABLE I. Cell-Free Amino Acid Incorporation into Rat Adrenal Protein Following Gastric Intubation of Dimethylnitrosamine.

After intubation (hr):	0	3	12
Sp ac (cpm/mg protein)	4312 ± 354 (11)	6500 ± 697 (5)	5180 ± 298 (5)
Difference from control (%)		50.7	20.1
		$p < .001$	$p < .05$

TABLE II. Cell-Free Amino Acid Incorporation in Rat Adrenal Protein Following Intraperitoneally Injected Celite or Dimethylnitrosamine (cpm/mg protein).

After ip injection	(hr): 2	12
Control (saline)	3730 ± 165 (5)	4020 ± 111 (4)
Celite	6140 ± 252 (4)	6280 ± 209 (4)
Difference from control (%)	64.6	56.2
	$p < .001$	$p < .001$
DMNA	6040 ± 392 (4)	6510 ± 515 (4)
Difference from control (%)	62.0	62.0
	$p < .001$	$p < .01$

inflammatory exudate, macrophage exudation and fibrin deposition. The irritant persists. DMNA injection is associated with an early exudation, whose course is more related to localized tissue injury (mesentery spread preparations show focal areas of cell death, whereas Celite treatment results in fibroplasia), whose time course coincides with the latter (12 hr) enhancement.[2]

It appears that DMNA exerts its effects on adrenal activity as a secondary manifestation of its direct action on the liver and kidney. This adrenal mediated stress response modulates the initial injury produced.

Summary. The effect of dimethylnitrosamine (DMNA) treatment *in vivo* on the rat adrenal gland was investigated using cell-free incorporation of leucine-^{14}C into protein as an index of metabolism. DMNA, by intraperitoneal injection or gastric intubation, resulted in an increased rate of amino acid incorporation into adrenal protein within 2 to 3 hr of administration. A second response to DMNA, which peaks at 12 hr, was found only after intraperitoneal injection of the drug. DMNA added to adrenal slices *in vitro* showed no stimulation of leucine incorporation into protein. Correlating the adrenal response to DMNA with the effect of diatomaceous earth (Celite) injection (ip) leads to the suggestion that the adrenal response to DMNA arises as a secondary manifestation of its direct action on other organs.

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