THE EFFECT OF PODOPHYLLOTOXIN, COLCHICINE, URETHANE, AND NITROGEN MUSTARD ON THE RESPIRATION OF NORMAL AND SUPRARENALECTOMIZED RAT LYMPHATIC TISSUE*

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During an investigation of the effect of the carcinoclastic compound, podophyllotoxin, on tissue respiration, we observed that injection of this compound into normal rats produced marked reduction of the respiration of spleen, lymph node, and thymus gland slices (1). Lymphatic tissue appeared to be particularly sensitive to podophyllotoxin, in sharp contrast to liver and kidney, which were not inhibited. This selective inhibition was clearly demonstrable only when the compound was *injected* into animals. The reduction of lymphatic tissue respiration when podophyllotoxin was added *in vitro* was not strikingly different from that of liver or kidney (2).

In order to determine whether this effect on lymphatic tissue is a unique property of podophyllotoxin or is common to other carcinoclastic agents, we have compared the activity of podophyllotoxin in this respect with three compounds of widely differing chemical structure, which have, nevertheless, the common property of inhibiting the growth of certain types of tumors. In this paper we present the results of experiments in which colchicine, urethane, and one of the nitrogen mustards, methyl-bis(β-chloroethyl)amine, have been compared with podophyllotoxin. These three compounds were chosen because, like podophyllotoxin, (1) they possess carcinoclastic properties; (2) they inhibit cell mitosis; (3) they depress hematopoietic activity (4–20). Moreover, they have the common property of provoking what Dustin (21) has called the "crise caryoclasique," the crisis of nuclear fragmentation, in the lymphoid system and thymus.

Published work on the effect of the compounds on tissue respiration is rather meager. In 1934 Lits first reported that colchicine inhibited the division of animal cells at metaphase (22). In the enormous body of literature on colchicine, comprising

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nearly 1500 references (23), there are surprisingly few studies on the effect of this interesting compound on enzymes and tissue metabolism. Boyland and Boyland (10, 13) observed that the respiration of several transplanted tumors in mice and rats decreased after colchicine injection, but that liver respiration was normal.

The effect of nitrogen mustards on enzymes and tissue metabolism has been somewhat more extensively investigated (3). One of us, in collaboration with Barron and others (24), found that lymphoid tissue was very sensitive to nitrogen mustards *in vitro*. The respiration of lymphatic tissue from animals *treated* with these compounds was not determined.

Urethane is of considerable historic interest because Warburg showed in 1910 that it arrested cell mitosis and division in fertilized sea-urchin eggs, without causing a marked change in the respiration of the eggs (25). However, the effect of urethane on the metabolism of lymphatic tissue has not been studied.

The results presented in this paper indicate that injection of nitrogen mustard and colchicine, as well as of podophyllotoxin, causes a selective reduction of the respiration of lymphatic tissue. Urethane was inactive against this tissue in the concentrations employed.

The rapid and selective action of these compounds on lymphatic tissues invites comparison with the early histological changes, especially involution of lymphatic tissue, which Selye considers characteristic of the alarm reaction or general adaptation syndrome (26). In their study of the toxicology of podophyllotoxin conducted concurrently with our experiments on tissue metabolism, Kelly, MacCardle, and Smith (27) did observe that some of the early manifestations of toxicity closely resembled phenomena which are characteristic of the alarm reaction. Colchicine, also, is known to elicit the alarm reaction (28, 29). It seems reasonable to assume that other compounds which act selectively on lymphatic tissues—such as the nitrogen mustards—might produce their metabolic effects on this tissue *via* the pathway which elicits the alarm reaction.

Selye has shown (26) that the alarm reaction is mediated through the suprarenal cortex, and depends on the release of corticoid hormones. When the suprarenal cortex is removed or destroyed, the stimuli usually effective—cold, poisons, etc.—no longer elicit the alarm reaction. To test the hypothesis that the inhibitory action of podophyllotoxin, colchicine, and nitrogen mustard might also require the participation of the suprarenal cortex, we have removed the suprarenal gland, and have studied the effect of this ablation on the metabolism of lymphatic tissues. We have compared the respiration of lymphatic tissues removed from suprarenalectomized animals injected with these compounds with that of normal animals, similarly treated. The results indicate a significant difference in the two groups, suggesting that the selective inhibition of lymphatic tissue respiration by these compounds is mediated by the suprarenal gland.

Methods

Weanling rats, which weighed 40 to 80 gm. and were maintained on a stock diet of Purina chow, were suprarenalectomized.[1] The animals were maintained in good condition after suprarenalectomy by administration of NaCl solution *ad libitum* instead of water. Studies were made on tissues of these animals 3 to 8 days after the operative procedure, which allowed sufficient time to insure complete disappearance of adrenocorticoid hormones.

Podophyllotoxin[2] was prepared as a 50 per cent solution in propylene glycol. Freshly prepared aqueous solutions of colchicine[3] and methyl-bis(β-chloroethyl)amine hydrochloride[4] were used. The urethane[5] was dissolved in the minimum amount of alcohol, then diluted with water to the desired final concentration. Sublethal amounts were injected subcutaneously into normal and suprarenalectomized rats as follows: podophyllotoxin, 1, 2, and 20 mg. per kilo body weight; colchicine, 0.5 and 2 mg. per kilo; methyl-bis(β-chloroethyl)amine hydrochloride, 2, 4, and 8 mg. per kilo; urethane, 1.5 gm. per kilo.

The animals were killed 24 hours after injection. The respiration of thymus and spleen from each animal was determined in Ringer-Krebs phosphate buffer in oxygen at 37°C. for 1 hour. Kidney tissue was chosen as a representative tissue of non-lymphatic nature, and similar determinations were made on kidney slices. The results are expressed in terms of Q_{O_2}, cubic millimeters of oxygen consumed per milligram dry weight per hour.

RESULTS

Relative Toxicities.—Suprarenalectomized rats proved to be considerably more sensitive to podophyllotoxin, colchicine, and the nitrogen mustard derivative than normal animals. Thus, all of 8 normal rats tolerated 2 mg. per kilo of podophyllotoxin, and 11 of 16 rats survived after administration of 15 mg. per kilo. However, only 7 of 10 suprarenalectomized rats tolerated 2 mg. per kilo of podophyllotoxin. Similarly, 11 of 12 normal rats treated with 8 mg. per kilo of methyl-bis(β-chloroethyl)amine hydrochloride survived, while only 5 of 9 suprarenalectomized rats could tolerate 4 mg. per kilo of this compound. Normal rats readily withstood 2 mg. per kilo of colchicine; 0.5 mg. per kilo was the maximum amount tolerated by the suprarenalectomized animals.

Effect of Injection of the Compounds.—As shown in Table I, 1 and 2 mg. per kilo body weight of podophyllotoxin had no effect on the respiration of normal spleen removed 24 hours after injection of the compound, whereas as little as 1 mg. per kilo reduced the respiration of normal thymus 22 per cent. As shown previously (1), 20 mg. per kg. reduced the respiration of normal thymus 65 per cent and that of normal spleen 44 per cent. After suprarenalectomy, 1 and 2 mg. per kilo body weight had no effect on the respiration of either spleen or thymus gland slices.

[1] We wish to express our thanks to Dr. Aldo P. Truant for his helpful guidance in this procedure.

[2] Podophyllotoxin, the S. B. Penick Co., New York.

[3] Colchicine, Merck, U.S.P.

[4] Methyl-bis (β-chloroethyl) amine hydrochloride obtained from Dr. Chester Stock at the Sloan-Kettering Institute for Cancer Research.

[5] Urethane, Merck, U.S.P. IX.

The respiration of normal spleen was reduced 16 per cent by 0.5 mg. per kilo of colchicine; 2 mg. per kilo caused an even more marked inhibition, 30 per cent. Similarly, normal thymus respiration was reduced 21 per cent by 0.5 mg. per kilo, and 42 per cent by 2 mg. per kilo. On the other hand, despite the much greater sensitivity of suprarenalectomized animals to colchicine, neither spleen

TABLE I

*Effect of Various Agents on the Respiration of Tissues from Normal and Suprarenalectomized Rats**

Tissue	Treatment of animals	No drug (Control)	Podophyllotoxin, *mg./kg.*			Colchicine, *mg./kg.*		HN_3,‡ *mg./kg.*			Urethane, *gm./kg.*
			1.0	2.0	20.0	0.5	2.0	2.0	4.0	8.0	1.5
Spleen	Normal										
	Q_{O_2}§ (average)	11.2	11.0	10.2	6.5	9.4	7.8	10.5		2.7	10.0
	Q_{O_2} (range)	8.3–13.4	9.8–13.4	8.3–11.0	2.6–9.2	6.8–12.5	5.1–9.0	7.6–13.1		0–4.3	8.4–10.9
	No. of animals	32	12	8	11	12	7	9		8	9
	Suprarenalectomized										
	Q_{O_2} (average)	9.9	9.8	10.2		9.8		7.9	6.8		
	Q_{O_2} (range)	7.3–11.6	7.7–11.1	8.6–11.2		6.1–12.1		5.9–8.9	4.9–8.1		
	No. of animals	18	14	7		14		13	5		
Thymus	Normal										
	Q_{O_2} (average)	10.9	8.5	8.4	4.4	8.6	6.3	8.7		0.9	10.3
	Q_{O_2} (range)	4.6–17.3	4.8–12.5	6.8–9.5	2.8–5.4	4.9–11.4	3.8–8.3	7.2–10.4		0–2.5	9.2–11.8
	No. of animals	32	11	8	6	10	7	9		8	9
	Suprarenalectomized										
	Q_{O_2} (average)	10.5	11.0	10.6		10.0		7.7	6.9		
	Q_{O_2} (range)	8.3–15.9	6.2–15.4	6.7–12.8		3.9–16.8		5.1–10.0	3.3–10.1		
	No. of animals	18	14	7		14		14	5		
Kidney	Normal										
	Q_{O_2} (average)	23.3			22.3		25.8	25.4			24.5
	Q_{O_2} (range)	18.0–27.5			19.0–23.6		21.6–30.2	21.4–27.9			20.5–28.0
	No. of animals	19			7		7	9			8

* The animals were killed 24 hours after injection of the compounds.

‡ Methyl-bis (β-chloroethyl) amine HCl.

§ Q_{O_2} = mm.³ oxygen consumed per milligram dry weight of tissue per hour, measured in oxygen at 37°C.

nor thymus respiration was affected by the injection of 0.5 mg. per kilo, the maximum tolerated amount.

The nitrogen mustard derivative, methyl-bis(β-chloroethyl)amine hydrochloride, had no effect on the respiration of normal spleen at a concentration of 2 mg. per kilo, but reduced the respiration of normal thymus 20 per cent. Eight mg. per kilo profoundly inhibited the respiration of both spleen and thymus of normal rats: in 8 rats, the average reduction of spleen was 76 per cent, and in one of these rats, the inhibition was complete; the average reduction of the respiration of thymus from these 8 rats was 91 per cent, and in 5 of these 8 animals, inhibition was either 100 per cent complete or very close to it. The

respiration of spleen slices from suprarenalectomized animals was reduced 20 per cent, and of thymus slices, 26 per cent, after the injection of 2 mg. per kilo. However, 4 mg. per kilo, the maximum amount tolerated by suprarenalectomized rats, reduced spleen respiration only 31 per cent, and thymus respiration, 34 per cent.

Urethane had no significant effect on normal rat spleen and thymus even at a concentration as high as 1.5 gm. per kilo body weight. Suprarenalectomized animals were not treated with this compound.

No reduction of the respiration of kidney slices from normal rats was observed with any of the compounds studied.

DISCUSSION

Our experiments indicate that colchicine and one of the nitrogen mustards, methyl-bis(β-chloroethyl)amine, are, like podophyllotoxin, very toxic to the lymphatic tissues of normal animals, and produce an inhibition of their respiration, readily demonstrable 24 hours after injection of the compounds. The effect on lymphatic tissue could be demonstrated at concentrations which did not influence kidney respiration. The concentration of urethane used, although a very high one, was without effect. These data suggest that studies of lymphatic tissue respiration might be a useful screening technique for evaluating possible therapeutic activity against tumors of lymphatic origin. The greater specificity shown after injection of the compounds as compared with exposure of tissue slices to the compounds *in vitro*, indicates that the former procedure would be more meaningful and revealing.

In accord with our working hypothesis, the selective reduction of the respiration of lymphatic tissue appears to be mediated through the suprarenal gland, for suprarenalectomy reduces the sensitivity of lymphatic tissues to these agents. Exact comparison with the results obtained on normal animals is difficult because the compounds studied are more lethal to suprarenalectomized animals. In all cases, the minimum lethal dose was smaller for suprarenalectomized animals than for normal ones. Nevertheless, even at highly lethal concentrations, lymphatic tissues of suprarenalectomized animals were inhibited to a smaller extent than corresponding tissues removed from normal animals. Thus, 1 mg. of podophyllotoxin per kilo reduced the respiration of normal thymus, but had no effect on thymus from suprarenalectomized animals; a concentration of colchicine which was close to the lethal amount for suprarenalectomized rats had no effect on the respiration of spleen or thymus from suprarenalectomized animals but inhibited that of both tissues from normal animals; a concentration of nitrogen mustard which killed nearly half the suprarenalectomized rats (4 mg. per kilo) had less effect on the respiration of spleen and thymus from the surviving animals than 8 mg. per kilo had in normal rats, despite the fact that normal rats readily tolerated this amount.

Leblond and Segal (28) demonstrated that 48 hours after the injection of

colchicine into rats, their thymus weight is reduced to 25 per cent of the normal value, and the weight of the suprarenals is nearly doubled. Suprarenalectomy prevents the decrease in thymus weight (28), but makes the animals three times as sensitive to colchicine, as indicated by mortality; to some extent, suprarenalectomy reduced the increased number of pycnoses observed in thymus after colchicine treatment (29). Clark and Barnes (30) found that cortin increased the resistance of animals to colchicine poisoning. Our results are in accord with these.

Although we observed that mortality is greater in suprarenalectomized rats than in normal rats following administration of methyl-bis(β-chloroethyl)amine, Anslow *et al.* (31) reported that massive doses of desoxycorticosterone acetate did not reduce the mortality of normal mice and rats treated with this compound. This is in sharp contrast with the results observed in colchicine poisoning, and the explanation for the difference is not apparent.

SUMMARY

The injection of podophyllotoxin, colchicine, and a nitrogen mustard derivative, methyl-bis(β-chloroethyl)amine, into normal animals causes a reduction of the respiration of the lymphatic tissues, spleen, and/or thymus. No effect was demonstrable on kidney, a representative tissue of non-lymphatic origin.

The degree of inhibition was considerably less in suprarenalectomized animals, suggesting that the inhibition is mediated by the suprarenal gland.

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