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Toxicology of Podophyllin.* (18746)

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When the colchicine-like effects of podophyllin were demonstrated(1,2), it was anticipated that the resin of podophyllin would be investigated in the treatment of cancer of the skin. Subsequent exhibitions of selective damaging effects of podophyllin and podophyllotoxin on mouse tumor cells *in vitro*, confirmed by *in vivo* studies in tumor bearing mice(3-5) stimulated the initiation of several clinical trials now in progress(6-8). Phillips, Chenowith, and Hunt(9) found the LD_{50} of podophyllotoxin to be 33 mg/kg for mice and 15 mg/kg for rats. Greenspan and Leiter(10) reported the maximum tolerated single dose of podophyllotoxin in mice to be approximately 30 μg/g.

The purposes of this investigation were: 1. To compare the toxicity of podophyllin with that of colchicine in mice. 2. To obtain additional toxicity data in rats on podophyllin and its components as a prelude to the clinical use of podophyllin in the treatment of cutaneous carcinoma(6). Podophyllin U.S.P. is a complex resinous mixture which contains: podophyllotoxin, the component principally responsible for the drug's curative effect on condyloma acuminatum(2,11,12); quercetin; and 2 recently isolated crystalline components, designated as alpha-peltatin(13) and beta-peltatin(14). Inasmuch as alpha-peltatin and beta-peltatin were unobtainable, our study was limited to podophyllin; podophyllotoxin and its 2 products of alkaline hydrolysis, namely, (1) picropodophyllin and (2) podophyllic acid; quercetin; and depodophyllotoxinized resin. Detailed descriptions of the aforementioned 6 materials are recorded elsewhere(12,15).

Method. Material for parenteral administration was prepared by dissolving each test substance, with the exception of picropodophyllin, in absolute alcohol. One cc of a one or 10% alcoholic solution of the test substance was added to 9 cc of a 10% gum acacia suspension or to a 0.5% methocel suspension. Picropodophyllin was suspended in water with methocel. Test materials for oral administra-

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TABLE I. Median Lethal Single Dose in Adult Albino Farm Mice, Averaging 20 g, of Drugs Tested, by Route of Administration.

Drug	No. of mice	Route	LD_{50}, mg/kg	S.E. LD_{50}
Colchicine	106	Subcut.	3.8	.34
Podophyllotoxin	117		24.6	.42
	150	Oral	90	2.2
Podophyllin	140	Subcut.	58	1.7
	130	Oral	68	4.8
Quercetin derived from podophyllin	158	Subcut.	97	3.7
	197	Oral	161	3.9
Quercetin derived from quercetron	218	Subcut.	99	2.5
	281	Oral	159	2.7
Picropodophyllin	188	Intraper.	280	22.7
Depodophyllotoxinized resin	171	Subcut.	522	11.1
	199	Oral	625	15.9
Podophyllic acid	167	Subcut.	799	35.3
	136	Oral	899	15.3

TABLE II. Median Lethal Single Dose in Young and Adult Rats of Podophyllin and Podophyllotoxin Administered Subcutaneously.

Drug	No. of animals	Young rats LD_{50} mg/kg	Young rats S.E. LD_{50}	Adult rats LD_{50} mg/kg	Adult rats S.E. LD_{50}
Podophyllin	74	18	1.4		
	159			24	1.1
Podophyllotoxin	84	8	.81		
	85			22	1.2

tion were incorporated in gum acacia or methocel suspensions. Autopsies were performed immediately after death. The following tissues were examined: skin, heart, lung, trachea, thyroid, parathyroid, tongue, stomach, small and large intestines, gonads, spleen, pancreas, brain, striated muscle, bone with marrow, and eye.

In Table I are summarized median lethal doses of substances tested for adult mice. The method used for obtaining the LD_{50} values and their standard errors was that of Litchfield and Fertig(16). In Table II are summarized comparative toxicity data on podophyllin and podophyllotoxin for young, recently weaned rats, averaging 28 g, and adult rats, averaging 150 g of the McCollum strain. Considered to be of interest were the following observations on symptomatology and pathology.

Podophyllin. Death occurred usually within 15 to 18 hours after parenteral administration or ingestion of fatal doses of podophyllin. Diarrhea, rapid and labored respiration and a dragging gait with hyperextended posterior extremities were observed in the majority of the animals during the 8 hours after dosing. At 9 hours there was a period of excitation, during which the animals ran wildly, and which was climaxed by spastic convulsions. This was followed by the terminal period, manifested by flaccidity, a dragging gait and slow, shallow, labored respirations. Microscopic studies were performed on 15 of the rats. The most constant finding was an acute enteritis. There were increased numbers of polymorphonuclear leukocytes in the submucosa of both small and large intestine and a striking breaking up of their nuclei so that small masses of deeply staining chromatin were found in profusion. Another prominent change was necrosis of the lymphoid tissue beneath the mucosa, usually affecting the central portion; again there were numerous aggregations of bits of chromatin. There was no apparent change in the mucosal epithelial cells; there did seem to be an increase in mitotic figures in their nuclei. Similar evidence of nuclear distintegration was present in lymphoid follicles of the spleen where again were found bits of chromatin. Increased mitotic activity was present in the epithelium of the tongue. There were no arrested mitoses in the skin.

Podophyllotoxin. Eight hours after administration of fatal doses of podophyllotoxin the animals were flaccid, their respirations were slow, shallow and labored. The terminal stage, in which they were somnolent or unconscious, lasted 4 to 7 hours, and death always occurred within 12 to 15 hours. Microscopically, 7 rats which had received a single dose of the toxin in this group were examined; all appeared normal. Four rats that had survived an initial LD_{50} dose were injected 2 weeks later with a fatal dose of podophyllotoxin; they showed the same changes in the intestine which the podophyllin treated rats had exhibited and they will not be detailed again. The spleens were also affected in the 4 rats that had received the 2 doses.

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TABLE III. Cumulative Effects of Podophyllin and Podophyllotoxin.

	No. of rats	Individual dose: Fraction of LD_{50}	Individual dose: mg/kg	Individual dose: No. per day	No. of days
Podophyllin	8	1/12	1.9	1	15, 105, 105, 105, 105, 105, 105, 105
	8	1/6	3.8	1	7, 7, 14, 15, 26, 31, 56, 105
	10	"	3.8	2	4, 4, 5, 5, 5, 6, 6, 6, 6, 9
	10	"	3.8	3	3, 3, 3, 3, 3, 3, 3, 4, 4, 4
	10	"	3.8	4	2, 2, 3, 3, 3, 3, 3, 3, 3, 4
Podophyllotoxin	5	1/10	2.2	1	5, 12, 38, 63, 105
	7	1/5	4.4	1	12, 15, 16, 50, 57, 63, 105
	10	"	4.4	2	2, 2, 4, 4, 6, 6, 6, 6, 30, 30
	10	"	4.4	3	3, 3, 3, 4, 4, 5, 5, 5, 6, 6
	10	"	4.4	4	1, 2, 2, 2, 3, 3, 3, 3, 3, 3

Picropodophyllin. Terminal signs were rapid respiration and tremors. Gait was normal until the terminal stage when flaccidity developed. Death occurred within 16 to 18 hours. No histologic observations were made.

Podophyllic acid. Death occurred in 17 to 18 hours and the terminal signs were similar to those produced by podophyllin. No histologic observations were made.

Quercetin. The terminal signs of poisoning from quercetin differed from those of podophyllin and podophyllotoxin. Within half an hour after injection the animals exhibited an abnormal gait in which there was a rapid swimming motion of the forepaws, following which all locomotion was accomplished by the posterior extremities with which they propelled themselves. During the next half hour their respirations became labored and they lay in a somnolent state on one side occasionally attempting to move. There was apparently some pruritus as all scratched their sides. Finally in an hour they became relaxed, flaccid, and unable to drag themselves forward. Following a period of 2 hours of rapid, deep breathing their respirations became shallow and slow and death occurred at 4 hours after the injections. A group of 10 adult rats, fatally poisoned by single injections of quercetin, was examined microscopically; no changes to account for death were found. The rapid lethal action of the drug may account for the absence of detectable alterations in the postmortem tissues.

Depodophyllotoxinized resin. The animals that died exhibited the following signs: fine tremors and a dragging, clumsy gait with their hind extremities hyperextended. Preceding death there were flaccidity, general weakness and slow, labored respirations. Death occurred usually 6 to 9 hours after injection of fatal doses. Microscopic examinations of 11 adult rats, fatally poisoned by single injections of depodophyllotoxinized resin, showed the intestinal damage we have already described in rats that died following injections of podophyllin. The spleen also showed nuclear fragmentation.

Colchicine. Thirteen rats fatally poisoned with single injections of colchicine were studied microscopically. Changes similar to those already described in rats poisoned with podophyllin were present in the intestine and spleen. In addition there was a marked increase in mitotic figures in the epitheliums of a number of tissues, namely, skin, tongue, esophagus, fore and hind stomach, intestine and gonad.

Cumulative effects of podophyllin and podophyllotoxin. The experiments summarized in Table III were designed to determine whether cumulative effects would result from the administration of daily, fractional, sublethal doses. When podophyllin and podophyllotoxin were administered subcutaneously in single daily doses of fractions of the LD_{50}, cumulative effects were produced in the majority only after prolonged periods. Cumulative effects were produced in shorter periods with similar single doses administered 2, 3 and 4 times a day.

Summary. 1. The median lethal doses of podophyllin, podophyllotoxin, picropodophyllin, podophyllic acid, quercetin from podo-

phyllin, quercetin from quercetron, podophyllotoxinized resin and colchicine were determined in adult mice. In this species podophyllin and podophyllotoxin respectively are approximately 1/15 and 1/6 as toxic as colchicine. 2. A comparison of the median lethal doses of podophyllin and podophyllotoxin in young and adult rats demonstrated a greater degree of toxicity for young rats. 3. Cumulative toxic effects were produced in rats by administering podophyllin and podophyllotoxin multiple times a day in individual doses that were in the range of 1/6 and 1/5 of the LD_{50}. 4. Observations on the symptomatology and or pathologic alterations of rats poisoned with podophyllin, podophyllotoxin, podophyllic acid, picropodophyllin, quercetin and depodophyllotoxinized resin and colchicine are presented. 5. In animals that succumbed to subcutaneous injections of colchicine there was an accumulation of arrested mitoses in the skin (positive Dustin reaction); in animals killed by subcutaneous injections of podophyllin the Dustin reaction was negative as there were no epidermal changes.

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Effect of Semliki Forest Virus on Rabbit Fibroma.* (18747)

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Certain neurotropic viruses have been shown to parasitize transplantable mouse tumors and cause necrosis of the tumors(1-3). Similar antagonism of a transplantable lymphoid tumor of chickens by Russian spring-summer and other neutrotropic viruses has been reported(4). It has also been demonstrated that Virus III causes suppression or early regression of the rabbit fibroma(5). The inhibiting action was demonstrated only when Virus III was inoculated at the same site as the fibroma virus. The present paper describes the results of a study of Semliki Forest virus (SFV) in tissue cultures of normal and tumor cells and the destructive effect of this agent on virus-induced rabbit fibromas.

Methods. Viruses. SFV was prepared as 20% suspensions of infected mouse brain. The intracerebral LD_{50} titer in 10 g mice varied from $10^{-7.5}$ to $10^{-9.0}$. Fibroma virus was obtained from Dr. M. H. D. Smith. This strain of virus was isolated by Dr. R. E. Shope and is similar in its characteristics to the original OA strain. Fibromas were produced on the abdomens of groups of New Zealand white rabbits by injecting 0.5 cc of a 10% extract of testicular fibroma tissue intradermally into 6 to 8 sites. Simultaneously, or at various intervals thereafter, 1.25 ml of a 20% SFV extract was injected intramuscularly. The course of fibroma growth was observed daily or every second day and the diameter and height of each growth was measured and recorded. The volume of each growth was estimated in cubic centimeters (cm^3) by multiplying the height $\times \pi$ radius^2. Weighed portions of fibromas were extracted, centrifuged and titrated intradermally on the abdomens of normal domestic rabbits, using 3 to 4 inoculations for each dilution and a 0.10 cc inoculum. The titer of a given extract was recorded as the Infectious $Dose_{50}$ (ID_{50}) (6). When fibroma virus titrations were made with extracts of fibroma that contained

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