Brain Concentration of Reserpine-H^3 and Its Metabolites in the Mouse.* (28855)

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The fate of reserpine in the brain and its pharmacological effects appear to have little correlation with tissue concentrations(1-4). Labeling with C^{14} in the trimethoxybenzoic acid portion of the molecule only served to confuse the issue, because of low specific activity and ready hydrolysis of the ester linkage(1,2). When tritium labeled reserpine became available, it was shown that the drug was present in the brains of guinea pigs for 72 hours(4). Thus, reserpine was present throughout and beyond the period of its pharmacological action. It was of interest to us to determine whether similar results would be obtained in another species, mouse, and if reserpine and its metabolites could be detected in the brain for even longer periods of time after a single dose of drug.

Methods. One hundred and two male CF1 mice weighing 20 to 30 g were used. Each animal received an intraperitoneal injection of 4 mg/kg of reserpine-H^3 having a specific activity of 7.8×10^5 d/m/mg. The drug was dissolved in phosphoric acid acidulated water. Observations were made of the pharmacological effects of the drug every 30 minutes for the first 3 hours after administration and regularly thereafter for the entire experimental period of 5 days. Animals in groups of 5 to 6 were sacrificed by decapitation at ½, 24, 48, 72, 96 and 120 hours after drug administration. The entire brain was removed from each animal, weighed and kept frozen until extracted. The weight of 5 or 6 mouse brains ranged from 1.62 to 2.36 g. All glassware was thoroughly cleaned with chromate cleaning solution. Reserpine and its metabolites were extracted using the method of Sheppard *et al*(1) with repurified acetone replacing the methanol used in the original method(2). Twenty ml of acetone was used for each gram of brain homogenate. Samples were kept at a low temperature, protected from light to prevent photo decomposition of the extracts. Fractions A, B, C and D were transferred directly into glass counting vials, dried, then placed in a desiccator for 12 hours over pellets of NaOH. The dry residues were dissolved in scintillation solution (toluene containing 5 g of PPO and 0.3 g of POPOP per l) and counted in a Packard liquid scintillation counter at 4°C. To reduce the counting error to 1.5% or less, at least 3500 counts were obtained. Additionally an internal standard was added to each sample which was recounted to correct for quenching. Total radioactivity recovery from an acetone reserpine-H^3 blank was 74.5% of which fraction B (reserpine) represented 99.1% of the total. Results are expressed as counts/min/g of brain.

Results. During the first 3 hours the animals exhibited classical symptoms of reserpinization: depression, ptosis, tremor, piloerection, hind limb extension, asymmetrical position, micturition, diarrhea, tachypnea and hypothermia. At 24 hours the depression was very marked, but this effect decreased progressively during the next 2 days, and the animals appeared normal 4 days post-injection.

As can be seen in Table I, the greatest recovery of reserpine occurred at ½ hour, but even at this time the amount was only 0.0036% of injected dose. Furthermore, the greatest recovery of reserpine plus its metabolites was at 24 hours with a total of 0.01% of the injected dose. However, even such small amounts of reserpine were capable of producing the known pharmacological actions of the drug. Table I also shows the decline in percentage of radioactivity in the reserpine fraction (B) with a corresponding increase

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in the other fractions during the 5 day experimental period. The magnitude of these changes expressed as counts/min/g of brain is illustrated in Fig. 1. Only slight changes are evident in Fraction B (reserpine) for the first 24 hours, thereafter this fraction declines as Fraction C (trimethoxybenzoic acid) and Fraction D (methylreserpate) increase in quantity. We have no explanation for Fraction A (non-specific lipids) which showed its highest concentration at 24 hours and declined thereafter. Paper chromatographic analysis failed to reveal the nature of the material(s) it contained. Furthermore, the total concentration was too low to fractionate on paper and would not move without streaming with the usual solvent systems.

Discussion. Our results showing the rapid penetration of reserpine into the brain confirm those of others(2,4). The results herein

TABLE I. Distribution of Reserpine and Its Metabolites in the Brain.

Fraction		% recovery of inj dose based on dpm	% distribution of radioactivity in the different fractions
30 min	A	.01517	10.31
	B	.11250	76.48
	C	.00767	5.22
	D	.01176	7.99
	Total	.14710	100.00
24 hr	A	.10380	43.99
	B	.08880	37.63
	C	.02997	12.70
	D	.01339	5.68
	Total	.23596	100.00
48 hr	A	.03253	26.45
	B	.06321	51.40
	C	.01409	11.46
	D	.01315	10.69
	Total	.12298	100.00
72 hr	A	.08838	51.09
	B	.06125	35.41
	C	.01162	6.72
	D	.01173	6.78
	Total	.17298	100.00
96 hr	A	.02197	24.25
	B	.05288	58.37
	C	.01428	15.76
	D	.00147	1.62
	Total	.09060	100.00
120 hr	A	.02707	23.50
	B	.04964	43.10
	C	.02256	19.59
	D	.01591	13.81
	Total	.11518	100.00

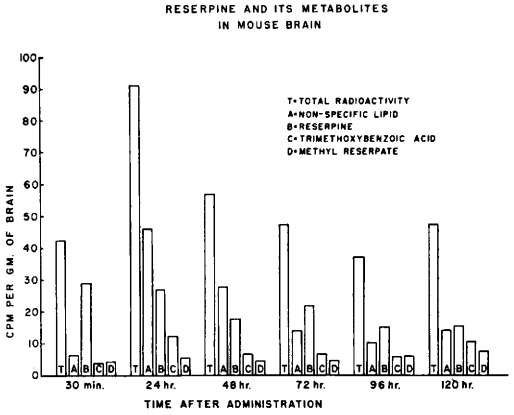

FIG. 1. Distribution of reserpine-H^3 and its metabolites in mouse brain as a function of time post-administration of 4 mg/kg of reserpine-H^3.

presented concerning the distribution of reserpine and its metabolites in the mouse brain agree with those reported by Sheppard *et al* (2,4) for both rats and guinea pigs. It is quite evident that in all cases very little of the injected dose of reserpine actually reaches the brain, but what does arrive there remains for at least 5 days. Moreover, the symptoms of reserpinization are still evident at 3 days, even though the amount of the drug is decreasing. There can be little doubt that reserpine releases both serotonin and catecholamines(5,6,7) from the brain. However, the low levels of these amines, the presence of reserpine and evidence of clinical signs of reserpinization still do not correlate to the degree that one can unequivocally state that the pharmacological effects are entirely dependent on release of the amines. We are inclined to agree with Sheppard and Zimmerman(8) that future work may establish the true mechanism of action of reserpine.

Summary. A study has been made of the reserpine H^3 content of mouse brain following a single intraperitoneal injection. There is a rapid rise in brain content in the first 24 hours and a slow decline in reserpine content thereafter. Trimethoxybenzoic acid and methylreserpate content of the brain increased as the reserpine decreased. The signs of reserpinization lasted for 3 days, while reserpine was present for 5 days.

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Virus-Like Particles in Human Leukemic Plasma.* (28856)

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Among the problems complicating study of the possible relation between viruses and human neoplasia are the lack of adequate techniques for detecting and isolating small numbers of virus particles from human tumors(1,2), and the inability to carry out transmission experiments in man comparable to those performed in animals(3). Even if a tumor is viral in origin, the level of virus in the tissues or in blood may be expected to be variable(4). Experimentally, injection of cell-free extracts from primary animal tumors generally results in a low incidence of disease transmission even in newborn, inbred animals. High yields of infectious particles that may infect other strains or species are often obtained only after repeated passage(5,6). In other instances, transplantable tumors, clearly produced originally by viral infection, have been shown to contain no(7) or at most a few infectious particles. A number of reports describing virus-like particles in human tumor material have been made(8-13; E. Frei, J. B. Moloney, A. J. Dalton, pers. comm.)

It appeared of interest to approach the problem of detecting oncogenic viruses in two steps, first to develop methods for isolating very small numbers of particles having the physical properties of viruses from human plasma or tissues, and second, to determine the possible infectivity of such particles in a variety of cell types grown in tissue culture and in several adult and newborn animals using more than one route of injection.

In this report 2 types of particles from human leukemic plasma are described, one of which resembles a previously described murine leukemia virus in morphology(18, density 1.18, T. E. O'Connor, R. F. Zeigel, and F. J. Rauscher, personal communication) but which differs from the murine virus in density. While the possibility that the particles are (a) viral and (b) causal remains to be examined, the correlation of distinct morphological entities with a pathological state is considered significant.

Methods. Whole-heparinized blood was collected and stored at 5°C until used. Cells and platelets were removed by centrifugation for 1 hour at 2700 rpm in a swinging-bucket rotor (International PR-2 centrifuge with head No. 855, 5281 $\times$ *g* at R_{min}, 11,239 $\times$ *g*

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