Affinity of chloroquine for bone

Previous studies (McChesney, Banks & Fabian, 1967; Varga, 1968; Grundmann, Vrublovský & others, 1972) have shown that chloroquine or its metabolites are preferentially accumulated in a number of tissues, depending on length of administration of the drug, dosages, and other factors. Quantitative recovery of all of the chloroquine administered to animals, however, has never been achieved. Either a significant amount of chloroquine or its metabolites may be sequestered in tissues not previously studied, or some of the metabolites may be undetectable by methods currently in use. In the course of validating autoradiographic studies using [^{14}C]chloroquine, we have examined quantitatively the distribution of radioactivity in the tissues of the chicken and the rat, in which we found a significant affinity of chloroquine or its metabolites for bone.

Two adult Sprague-Dawley (Carworth) rats, a male and a female, ~300 g each, two 8 day old rats of the same strain, ~13 g each, and two 1 day old leghorn chickens, 32 g each, were given intraperitoneally 0·5 μCi (96·4 μg) of [3-^{14}C]chloroquine (New England Nuclear) dissolved in 0·1 ml of 0·9% NaCl. Thirty min after injection, the animals were killed, the soft tissues were homogenized in water, and a sample of each homogenate was extracted with heptane according to Fitch (1969). The bones were dissolved in concentrated HCl, the acid neutralized with NaOH, and finally, a sample of the mixture was extracted with heptane (Fitch, 1969). To evaluate the effectiveness of the extraction, a known quantity of [3-^{14}C]chloroquine was added to samples of the various preparations before extraction. The recovery of the added ^{14}C was 98·1 $\pm$ 0·77% (mean $\pm$ s.e.) in 16 trials. A toluene-based liquid scintillation cocktail (Liquifluor, New England Nuclear) was used to count the heptane extracts. The counting error did not exceed 3%.

In addition, two 1 day old chickens and two 8 day old rats were subjected to whole body autoradiography according to Ullberg (1962). These animals were injected with 5 μCi of [3-^{14}C]chloroquine and were killed 30 min later; then they were frozen on dry ice and stored at $-70°$ before sectioning at $-4°$. Sections 40 μm thick were placed against RP Royal X-Omat X-ray film and exposed for one week. The films were then developed in Pix developer for 5 min and cleared in acid fixer (Kodak). As an additional control procedure to evaluate negative chemography, another set of X-ray films were fogged by a 1 s exposure to light before placement of the tissue sections (Rogers & John, 1969).

In the quantitative studies, the total recoveries of radioactivity, expressed as a percentage of the injected dose, were 52 and 55 for the adult rats, 94 for one of the 8 day old rats, and 59 and 82 for the 1 day old chickens. Degradation of chloroquine to products not extractable into heptane under our conditions is probable and could account for the incomplete recoveries (McChesney, Conway & others, 1966). Each of the individual tissues contained radioactivity. The adrenal glands contained the largest amount per gram wet weight, followed in rank order by spleen, liver, kidney, lung, heart, skeletal muscle, and bone. With the exception of bone, our findings approximate to those of McChesney & others (1967).

The quantitative recovery of radioactivity in selected tissues is illustrated in Fig. 1. A major fraction of the injected dose of radioactivity was found in the gastrointestinal tract; since the drug was given intraperitoneally, however, residual radioactivity from the injection may be included on this fraction.

The relatively large amounts of radioactivity in skin, bone, and lungs of the 8 day old rat were confirmed in the second 8 day old rat, although the data are not shown because a faulty injection of the [^{14}C]chloroquine prevented an accurate measurement

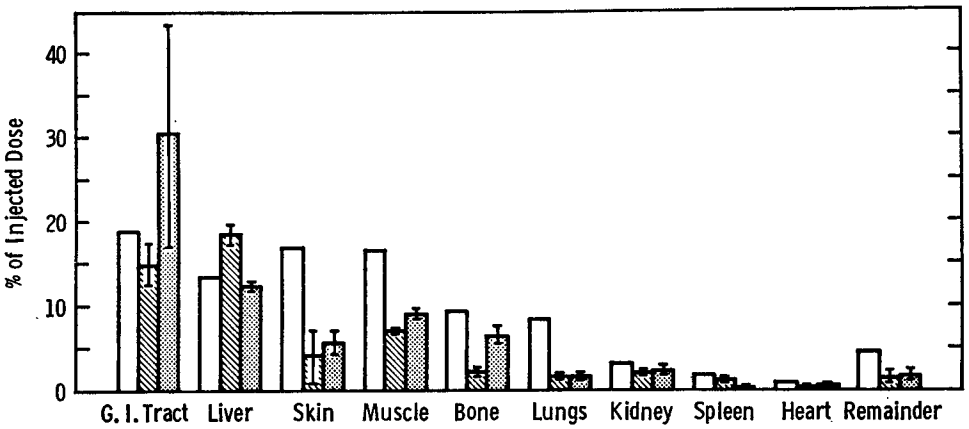

FIG. 1. Tissue distribution of radioactivity. Open columns, one 8 day old rat; hatched columns, adult rats; stippled columns, 1 day old chickens. The vertical lines represent the actual values obtained from two animals.

of the dose. Nevertheless, of the radioactivity recovered from the tissues of this animal, 17·2% was in skin, 11·6% was in bone, and 8·3% was in the lungs. Such large amounts of radioactivity in skin, bone, and lungs of the young animals may be due to differences in metabolic demands and tissue composition of growing animals or to the fact that a larger dose relative to body size was injected.

Thus bone emerges as a potentially significant storage organ for chloroquine or its metabolites which may account in part for the low recoveries of chloroquine in earlier studies. Furthermore, it is reasonable to postulate that chloroquine stored in bone represents not only a significant pharmacologic factor in the maintenance of chloroquine concentrations in plasma after discontinuance of the drug (Berliner, Earle & others, 1948), but also may exert an effect on calcium metabolism (Hunt & Yendt, 1963).

The results of the autoradiographic studies were essentially in agreement with the work of Lindquist & Ullberg (1972); all the major organs except bone were observed to contain radioactivity. In neither study was radioactivity in bone detected. Presumably this failure is due to the inability of β-particles from ^{14}C to penetrate the dense bony matrix and reach the emulsion layer of the X-ray film. No loss of latent image due to negative chemography by bone was observed in our control X-ray films.

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γ-Aminobutyrylcholine and GABA receptors on primary afferents in the frog spinal cord

There has recently been considerable discussion on the pharmacology of γ-aminobutyrylcholine (GABACh). Although the structural similarities between GABACh and the γ-aminobutyric acid (GABA) antagonists, bicuculline (Howells, 1971) and *N*-methyl bicuculline (Pong & Graham, 1972), suggest that GABACh might intereact with GABA receptors, most pharmacological studies, as reviewed by Johnston & Curtis (1972), have found GABACh to have little GABA-like activity (Honour & McLennan, 1960; Hance, Winters & others 1963; Curtis, Phillis, & Watkins, 1961; Krnjević, 1964; Crawford & Curtis, 1964). Bowery & Brown (1972) tested GABACh on sympathetic ganglia which possess both acetylcholine and GABA receptors and found that GABACh has little acetylcholine-like activity but strong GABA-like activity. Since the GABA-like activity was blocked by cholinesterase inhibitors, these investigators concluded that the GABA-like activity principally results from the formation of free GABA by the hydrolysis of GABACh. I have noted a less potent GABA-like activity of GABACh on primary afferent fibres which is entirely resistant to cholinesterase inhibitors. Thus these results suggest that GABACh can interact with the GABA receptors on primary afferents or that, if hydrolysis occurs, the enzyme involved is resistant to cholinesterase inhibitors (cf. Curtis & others, 1961; Holmstedt & Sjöqvist, 1960).

The effect of drugs on the membrane potential of primary afferent fibres in the frog isolated spinal cord (*Rana pipiens*) was measured by sucrose gap recording (Barker, Nicoll & Padjen, 1975). The Ringer solution contained either 20 mM $MgSO_4$ or 1 μM tetrodotoxin to block indirect synaptic effects. All experiments were at room temperature (20°). Thin-layer chromatography demonstrated that the GABACh contained no free GABA. Butanol–acetic acid–water (200 : 30 : 75, by vol.) was used as solvent. The chromatographs were exposed to iodine vapours for visualizing the spots. The GABACh was prepared in Ringer solution at the beginning of each experiment, to minimize the possibility of spontaneous hydrolysis.

In all 15 preparations GABACh exerted a depolarizing action on the primary afferents. The action of GABACh and GABA, unlike that of acetylcholine and carbachol, was not blocked in the presence of $MgSO_4$ or tetrodotoxin. The responses in Fig. 1A were obtained in a preparation in which synaptic transmission was blocked with tetrodotoxin. Both GABA and GABACh depolarize the dorsal root, while carbachol, which depolarizes in normal Ringer solutions, has a slight hyperpolarizing action in a Ringer containing 20 mM $MgSO_4$. The GABACh response often lasted up to 10 min after the application, while the GABA response subsided quickly after the application. The potency of GABACh relative to GABA was 0·05. The dose response curves for GABA and GABACh are shown in Fig. 1B.