This is less than the charge in the intact rat myocardium, but approximates the charge in the myocardium after a regime of exercise [1]. After exposure of the cells to allopurinol, the value is increased and approaches that of the intact myocardium.

The concentration of allopurinol (5×10^{-4} M) used in the heart cell experiments is approximately the same as the concentration of allopurinol and its primary metabolite, oxypurinol, found in the plasma of patients treated with the drug [13]. After exposure of the F-cells to this level of allopurinol, both the uptake of purines and the energy charge were increased over control values, in contrast to the M-cells in which uptake was not affected appreciably. An increased availability of purine bases for recycling to nucleotides by the inhibition of xanthine oxidase [14] probably does not explain these results, since the enzyme has not been found in human skin fibroblasts [15] and may not occur in heart F-cells either. If this enzyme is absent in F-cells, the stimulation of nucleotide formation by allopurinol is more difficult to explain. It may be speculated that an alteration in the activity of other enzymes is involved. The activity of 5′-nucleotidase is apparently not changed by allopurinol [15]. A small but significant accumulation of IMP and a decrease in GMP occur in the intracellular fraction of F-cells exposed to allopurinol, suggesting an inhibition of the conversion of IMP to GMP. This may allow a greater conversion of IMP to AMP and then to ADP and ATP. Such an effect may occur as a result of complex interactions of allopurinol and its metabolites with cellular enzymes, which may lead to changes in feed-back inhibition of *de novo* synthesis, availability of phosphoribosylpyro-phosphate, and modification of various reaction rates [15, 16].

In summary, it was found that uptake and retention of adenine were greater than of hypoxanthine in myocardial cells and fibroblasts isolated from the same heart ventrical preparation. Exposure to allopurinol increased uptake of both substrates by fibroblasts only, and increased retention of hypoxanthine metabolites in both cell types. In addition, the ratio of ATP + ADP to AMP was increased 1.5 and 1.9 times the control values in adenine-labeled fibroblasts and myocardial cells respectively.

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Pilot study on the distribution of ^{14}C-labeled methaqualone in the rat brain

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Methaqualone [2-methyl-3-*o*-tolyl-4-(3H)-quinazolinone] is a hypnotic/sedative, chemically unrelated to other groups of sleep-inducing drugs, or to any of the categories of major tranquilizers. It was first synthesized in 1951 by Kacker and Zaheer [1], and since then its clinical efficacy has been widely investigated and found favorable [2–6]. Even cases of intractable insomnia have been shown to be responsive to the compound [7]. Unfortunately, during the past few years methaqualone has become a favorite of the drug-using subculture [8–10], which fact eventually led to its classification by the Food and Drug Administration as an addictive compound included in Schedule II. The addictive potential of the drug, however, is less than that of barbiturates [11, 12].

Both the free base and its salts are readily absorbed from the gastrointestinal tract, reaching peak blood concentrations 1–2 hr after ingestion [13, 14]. The compound is distributed throughout the body, but because of its high lipid solubility, it is preferentially stored in fatty tissues [15]. While it is metabolized in the liver by microsomal enzymes, the degradation products enter the hepatobiliary circulation [16]; therefore, its elimination from the body is somewhat protracted, with a half-life of about 20 hr.

The exact site of action of methaqualone in the brain is unknown, but claims have been advanced that it must influence different centers than either the barbiturates or glutethimide [17]. The present pilot study was undertaken to elucidate, on the gross morphological level, the probable

site(s) where the drug might have its primary effect on the brain.

MATERIALS AND METHODS

2-[^{14}C]methaqualone, with a specific activity of 2.41 μCi/mg, was kindly supplied by William H. Rorer, Fort Washington, PA, Inc. The powder was dissolved in polyethylene glycol (PEG-400 Carbowax, Fisher Scientific Co., Houston, TX). A 30 μCi dose (~ 44 mg/kg) in 0.5 ml PEG-400 was given by stomach tube to a total of ten male WFu rats (obtained from Microbiological Associates, Bethesda, Md.), weighing approximately 350 g each. This dose caused a rapid hypnotic effect with an onset of about 5 min and a duration of somewhat over 3 hr. After the intubation, the animals were anesthetized with ether at 1-, 2-, 3-, 6- and 24-hr intervals, two animals being used for each period. (One animal for the 24-hr period received the complete dose and the other only one-half of the dose, i.e. 30 and 15 μCi respectively. The counts from this second 24-hr animal were multiplied by 2, and the resultant values, being virtually identical to those from the full-dose animal, were included in the averages.) While under anesthesia, the carotid arteries were prepared for cannulation, and the arch of the aorta was ligated. In this way, the whole blood supply of the brain was cut off. Through the cannulated carotids, 100 ml of normal saline was perfused through the brain, in order to remove the blood from the vessels. The vena cava was cut open, and the perfused saline with admixed blood left the body through this opening. (This technique is somewhat similar to that of Thompson *et al.* [18], used for the perfusion of the isolated rat brain.) Simultaneously with the above-described maneuver, whole blood was collected by cardiac puncture.

The brain of each animal was removed intact from the skull, together with the cervical medulla, care being taken to dissect out the optic nerves and the pituitary gland. Microscopic examination of the tela chorioidea showed it to be completely devoid of red corpuscles. The individual brains were submerged for hardening in Tris-buffered 10% formaldehyde for 24 hr. The central nervous system (CNS) was split in the middle, and each half was dissected into ten portions. The following parts were separated: (1) cortex; (2) cerebellum; (3) olfactory bulb; (4) corpus callosum; (5) thalamus and hypothalamus; (6) midbrain: peduncle with colliculis; (7) reticular formation of the medulla oblongata; (8) white matter of the pons and of the medulla oblongata (mainly, the fimbriae pontis transversae and the tractus pyramidalis); (9) optic chiasm, optic nerve and portions of the optic tract; and (10) cervical medulla. Due to the small dimensions of the pituitary gland, they were not divided in two. During this procedure, it was noted from which side of the brain the tissue originated.

Samples of 50–100 mg of nervous tissue, dried by blotting on tissue paper, were transferred into pre-weighed liquid scintillation counting vials, solubilized in 1 ml of Soluene 100 (Packard Instrument Co., Downer Grove, IL.) and exact tissue weights established. Radioactivity was determined by liquid scintillation spectrometry (Packard TriCarb, model 3000), using 10 ml Aquasol (New England Nuclear Co., Boston, MA) per sample. Counting efficiency was approximately 60 per cent.

The results are presented in Fig. 1. Although a slight but consistent elevation was found in the quantity of the drug

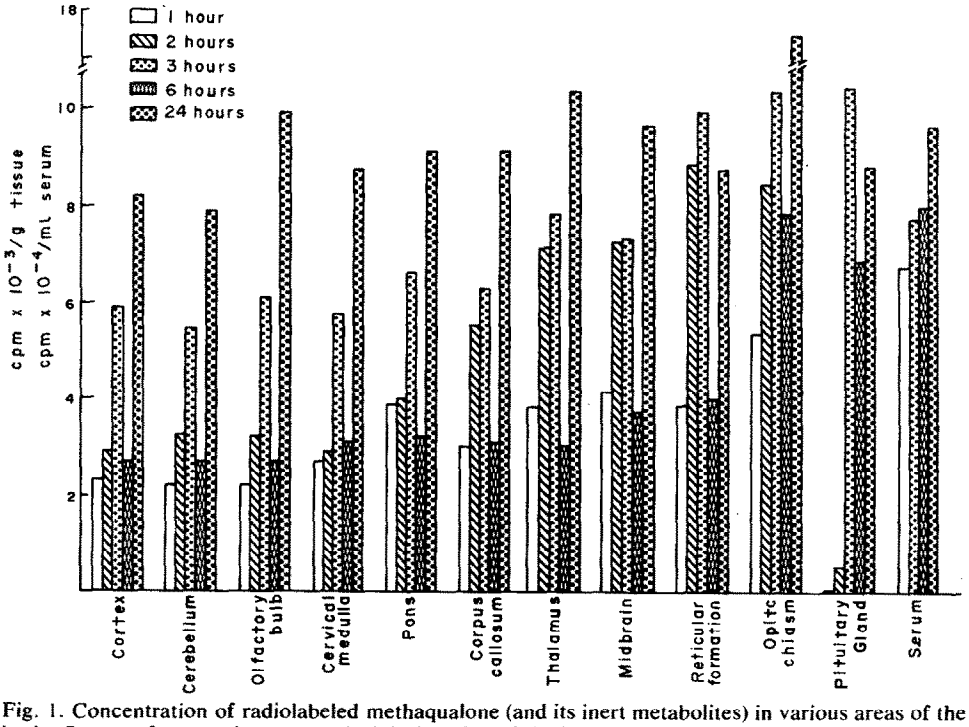

Fig. 1. Concentration of radiolabeled methaqualone (and its inert metabolites) in various areas of the brain. See text for complete anatomical designation of the investigated areas. The ordinate represents specific activity based on wet weight of tissue, whereas the different columns correspond to the time elapsed after administration of the labeled drug at 0 hr. It is assumed that the radioactivity at 24 hr was due to physiologically inactive metabolite(s) of the parent compound (see text for further details). Serum levels are given, but note that the specific activity is approximately one order of magnitude higher than that of the tissue levels. The presumed metabolite(s) in the serum shows a continuous increase during the experimental period. All bars represent the mean of four values, two brain halves of two animals each, with the exception of the pituitary gland which was not divided in two. The values for the sera are duplicate determinations from two animals for each time period. Examples for standard deviation are given in the text.

in the right as compared with the left brain, this did not reach more than 10 per cent. The deviation between animals for the same time interval was less than 4 per cent. The values, therefore, are represented as averages of counts/min from two brain halves of each of two animals. Standard deviations were calculated and corrected for 95 per cent confidence, using t-values to compensate for the tendency of small samples to underestimate variability. The results were too slight to represent graphically in Fig. 1. As an example, however, values for the cortex are given (all in cpm $\times 10^3$/g of tissue): 1 hr, 2.2 ± 0.117; 2 hr, 2.8 ± 0.118; 3 hr, 4.6 ± 0.235; 6 hr, 2.6 ± 0.123; and 24 hr, 8.2 ± 0.245.

It is evident that in the cortex, cerebellum, olfactory bulbs, cervical medulla, and the external portions of the pons and the medulla oblongata the highest concentrations are reached at 3 hr after intubation, and by 6 hr the levels of the drug in these tissues decline to approximately the same as those at 1 hr. What is noteworthy is that the tissue concentrations after 24 hr are almost twice those of the 3-hr peak. The simplest explanation for this phenomenon would be that a physiologically inactive metabolite of the parent compound is preferentially accumulating in the brain. Methaqualone in the corpus callosum, thalamus/hypothalamus, midbrain and reticular formation reaches much higher concentrations at an earlier time (2 hr), and also its absolute quantities, i.e. the peaks at 3 hr become higher than in the first five regions mentioned.

The optic chiasm and the pituitary gland show a somewhat aberrant pattern of drug accumulation. Of all brain parts investigated, these exhibit the highest drug concentrations. At 1 hr, there is almost as much methaqualone in the chiasm as there is after 2 hr in the corpus callosum; on the other hand, in the pituitary there is a definite time lag observable in the uptake. Notwithstanding this time lag, however, at the 3-hr period, the concentration in the pituitary is the highest of all the brain tissues examined. The serum levels, which, it should be noted, are one order of magnitude higher than the values for the tissues, show a continuously increasing pattern throughout the 24-hr period, in contrast to the peak tissue concentrations, which are reached at 3 hr and which decrease considerably by 6 hr. This would seem to indicate that by the third hr a metabolite appears, first accumulating in the serum and eventually penetrating the brain and giving rise to the high radioactivity levels measured there at 24 hr.

While investigating the distribution of different psychopharmaceuticals in the brain, several authors followed similar procedures to those we have employed, i.e. dissecting the brain into its major functional units and subsequently determining the concentration of the compound in each region. Unfortunately, in a number of studies (cf. Sanders [19]), the brains of the animals were not perfused prior to measuring drug concentrations; thus, one cannot define precisely how much of the given compound was indeed in the tissues and how much of it was in the blood trapped in the vessels. Since from 80 to 95 per cent of the various tranquilizers, similarly to methaqualone, are plasma protein bound [13], and thus only a small proportion is available for their tissue uptake, considerable distortion of results may ensue if blood contamination of the brain is not avoided. Furthermore, the serum concentrations of even unmetabolized methaqualone measured before 3 hr were shown to be one order of magnitude higher than those found in the brain, indicating that, when the trapped blood from the brain is not quantitatively eliminated, erroneous conclusions could be reached.

Both in experimental animals and in man, peak methaqualone blood concentrations occur from 1 to 2 hr after oral ingestion of the drug [20]. In our investigations, we found that the measured radioactivity showed a tendency toward continuous increase during the whole experimental period. In previous studies, after the peak concentration had been reached, a rapid fall in the serum levels of the drug was found at first, followed by a slower decline [20]. This discrepancy between results reported earlier and our own can be explained by the fact that we measured total radioactivity which, by necessity, included both the activity of the parent compound and of all its metabolites*. Other investigators have used gas chromatographic, spectrofluorometric or thin-layer chromatographic techniques, after various extraction methods. Such techniques are capable of specific identification of the drug and its major metabolites, but they probably do not permit assessment of certain small metabolic fragments. That the measured high activity at 24 hr originated from a physiologically inactive metabolite is borne out by the known duration of action of the drug, which is about 8 hr in humans, and also by our observation of the experimental animals, which behave in a completely normal and alert manner by this time.

From the distribution pattern presented, it becomes evident that methaqualone is taken up most rapidly and in greatest quantity by the phylogenetically oldest portions of the brain, although the olfactory bulbs do not conform to this statement. Activity in the reticular formation is particularly noteworthy. In contrast, the cortex shows relatively low levels of the drug, which may serve as indirect evidence that methaqualone does not primarily produce cortical inhibition in its sleep-inducing effect.

The observation of a time lag in the accumulation of the drug in the hypophysis could possibly be explained as a consequence of the meager blood supply to the pituitary gland. The very high concentration of methaqualone found in the optic nerve and the chiasm is interesting, but no explanation can be offered at the present time to account for this site specificity.

In summary, we have investigated the distribution of radioactive methaqualone in the rat brain after oral administration of the drug. Peak tissue concentrations were found 3 hr after intubation. The highest concentrations appeared in the optic chiasm, pituitary gland, and reticular formation of the medulla oblongata. Serum concentrations were approximately one order of magnitude higher than those found in the brain. An apparently inert metabolite was found at 24 hr in concentrations which were double those of the peak tissue levels of the compound, both in the brains and in the sera.

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* Murata and Yamamoto [21] reported that ring cleavage, resulting in the splitting off of a 2-carbon fragment, is an important metabolic pathway for methaqualone, a finding which has been confirmed [22]. In the material used in our study, the ^{14}C-label was at position 2, exactly the site which would be cleaved as a 2-carbon fragment in this process. Such low molecular weight fragments can easily accumulate in serum and tissue.

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Inhibition of nucleoside uptake in HeLa cells by nitrobenzylthioinosinate*

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Transport-specific elements of the plasma membrane mediate the passage of nucleosides into animal cells [1–3]. Various S^6-derivatives of 6-thiopurine nucleosides are potent inhibitors of nucleoside transport; these inhibitions are specific in that uptake of sugars, amino acids and nucleobases is not inhibited. Nitrobenzylthioinosine, the best known member of this group, binds tightly, but reversibly, to specific sites in the plasma membrane; occupancy of these sites in human erythrocytes [4] and HeLa cells [5] results in inhibition of nucleoside transport. In this report, we describe the synthesis of the 5′-monophosphate derivative of nitrobenzylthioinosine (nitrobenzylthioinosinate) and the inhibition by this compound of nucleoside uptake in HeLa cells.

Chemical synthesis. Thioinosinate (6-thio-9-β-D-ribofuranosylpurine 5′-phosphate) was prepared from 6-thioinosine (10 m-moles) by reaction with 3 ml phosphorus oxychloride and 30 ml trimethyl phosphate at 0° [6]. After 2 hr, the reaction was terminated by the addition of 200 ml of cold anhydrous diethyl ether, followed by vigorous stirring for 5 min; after allowing the syrup to settle, the ether phase was discarded. Cold triethylammonium bicarbonate (1 M, pH 7.5) was immediately added to the syrup until the pH of the resulting solution was 7.0. After 2 hr at 0°, the reaction mixture was dried under vacuum at 30° and co-evaporated three times with water, slurried with 10 g silicic acid (60–200 mesh) in water and finally dried under vacuum. The dry product was then added to the top of a dry-packed silicic acid column (3 × 30 cm) and eluted with the following solvents: 800 ml acetonitrile followed by 500 ml each of 18, 22, 25 and 30% 1 N ammonium hydroxide in acetonitrile. Eluate fractions (10 ml) containing thioinosinate [identified by thin-layer chromatography (t.l.c.) on cellulose in isobutyric acid–NH_4OH[conc]–water (66:1:33, v/v)] were freeze-dried and the product so obtained (yield 72 per cent) was judged to be authentic on the basis of the u.v. absorption spectrum [7], chromatographic behavior and susceptibility to hydrolysis by snake venom 5′-nucleotidase (Sigma Chemical Co., St. Louis, MO).

Thioinosinate (0.43 m-mole) was added to a solution of α-bromo-*p*-nitrotoluene (0.50 m-mole) in 2.4 ml of 2.3 M NH_4OH, 8.0 ml of 1,4-dioxane and 4.0 ml tetrahydrofuran. After 1 hr at 20°, the reaction mixture was dried under vacuum at 30°, adsorbed onto 10 g of silicic acid and added to a silicic acid column (2 × 40 cm) as above. The column was eluted with 400 ml acetonitrile followed by 100 ml each of 18, 20, 22 and 25% 1 N NH_4OH in acetonitrile. Eluate fractions (10 ml) containing nitrobenzylthioinosinate [recognized by t.l.c. on cellulose in 95% ethanol–1 M ammonium acetate (7:3, v/v)] were pooled and freeze-dried. The product (yield, 65 per cent) had the following characteristics: u.v. (H_2O): max 287 nm (ε,23,117), min 240 nm (ε 3,986); n.m.r. (Me_2SO-d_6): 4.75 to 3.88 (m, s, 2′, 3′, 4′, 5′ sugar protons), 4.75 (s, 2, benzylic protons), 6.01 (d, 1, J = 6 Hz, $C_1'H$), 7.72 and 8.13 [d (pair), 4 phenyl protons], 8.82 and 8.74 ppm [s (pair), 2, C_2H and C_8H]. Treatment with 5′-nucleotidase converted the product to nitrobenzylthioinosine. Analysis (Microanalytical Laboratory, University of Alberta, Edmonton, Alberta, and Schwarzkopf Microanalytical Laboratories, Woodside, NY) of the monoammonium salt of $C_{17}H_{21}N_6O_9PS \cdot 3\,H_2O$ as calculated was: N, 14.7; P, 5.44; S, 5.61; and as found: N, 14.27; P, 4.96; S, 5.75.

Nucleoside uptake. Replicate monolayer cultures of HeLa S3 cells were prepared as previously described [8];

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