and catalytic rate constants for ethanol and hexanol are included for comparison. As with saturated and unsaturated monohydric alcohols [5], the K_m values of the diols decrease with an increase of their carbon chain length. While 1,2-ethanediol is a relatively poor substrate, 1,5-pentanediol is as good as ethanol.

The catalytic rate constants increase with an increase of chain length until a virtual plateau is reached at 1,4-butanediol, where the values for horse and our preparation of the human ADH were *ca.* 180/active site/min and 11/active site/min, respectively; these values are determined by the rate of NADH dissociation from both enzymes [8, 9]. Diols of short chain length are interconverted at high concentrations with low catalytic rate constants in comparison with monohydric alcohols of the same chain length. There is a great similarity between horse and human ADH in catalysis of dehydrogenation of diols.

The distance in carbon atoms by which the hydroxyl groups are separated is another feature which appears to determine the effectiveness of diols as substrates. Thus, 1,3-propanediol seems to be a better substrate than 1,2-propanediol: its K_m is less than that of 1,2-propanediol and its turnover number is greater; an analogous comparison holds for 1,4-butanediol and 1,3-butanediol. This comparison, however, is not exact because in the first case both hydroxyls are primary alcohol groups, while in the second case one of the hydroxyl groups is a secondary alcohol.

The current preparation of human liver ADH is the same as that used by us previously for determining substrate specificity with saturated and 2-enoic alcohols [5]. The results obtained here with the diols, therefore, are directly comparable with those obtained previously.

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Effects of allopurinol on purine metabolism in cultured heart cells

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The release of myocardial adenosine appears to be involved with the adjustments of coronary blood flow to the metabolic requirements of the heart [1]. The loss of purines from the releasing cells must be replenished by either *de novo* synthesis or reutilization (salvage synthesis), or both. The salvage pathway is quite active in the heart [1], and in the presence of allopurinol, a xanthine oxidase inhibitor, it has been postulated that the nucleotide polyphosphate pool is increased during or after periods of oxygen depletion [2, 3]. The present study is concerned with the effects of allopurinol on the uptake and release of adenine and hypoxanthine and their metabolites, and on the status of the intracellular purine nucleotide pool in cultured fibroblasts (F-cells) and beating myocardial cells (M-cells) segregated from the same neonatal heart preparation.

Methods. Hearts from 1- to 2-day-old Sprague–Dawley rats were collected in the sterile nutrient medium employed by Mark and Strasser [4]. The same nutrient medium was used subsequently for the incubation of the isolated heart cells. The apical half of each ventricle was minced and transferred to a flask to which was added 2.5 ml per heart equivalent of 0.125% trypsin in Ca^{2+}- and Mg^{2+}-free Hank's basic salt solution. Gentle stirring was continued for 20 min at 37°, after which the supernatant was discarded. Cells were collected by low-speed centrifugation (1000 rev/min, 4 min) at room temperature after three subsequent periods of digestion of 15 min each in the presence of the same volume of trypsinizing solution. A nearly total maceration of the tissue resulted after this treatment. The sedimented cells were resuspended in the nutrient medium, combined, and 2-ml aliquots layered in a sterile centrifuge tube on 10 ml of nutrient medium containing 2% ficoll (mol. wt 400,000). A low-speed centrifugation (1000 rev/min, 4 min) at room temperature resulted in the sedimentation of intact cells in the loose pellet. The supernatant, containing cellular and other debris, was discarded. The pelleted cells were then washed once by gentle suspension in fresh medium and centrifugation. This preparation represented a purified mixture of viable F- and M-cells obtained from the trypsinized ventricles. The passage through the ficoll–nutrient medium mixture did not affect the viability of the cells and the ability of the myocardial cells to continue beating for at least 4 weeks, and it had the added advantage of reducing initial bacterial contamination.

Cultures enriched with either F-cells or beating M-cells were obtained from the same heart tissue preparation by a differential rate of attachment [5]. Primed culture plates were inoculated with the above mixed cell population containing approximately 2×10^6 cells, and the cultures were incubated in a water-saturated 5% CO_2–95% air incubator at 37°. The F-cells were found to attach to the surface of the culture plate more rapidly than the M-cells. Optimally, the unattached M-cells could be aspirated after 60 min of incubation. Fresh nutrient medium was added to the attached F-cells, and the M-cells in the aspirated medium were concentrated by centrifugation and replated in fresh medium. The nutrient medium was replaced after 24 hr, and the cultures were assessed for morphology and rate of contraction. After 3 days the F-cell cultures were confluent and were essentially pure (99 per cent or better). M-cell cultures represented an enrichment from about 60

Table 1. Distribution of label in heart M-cell and F-cell cultures*

Fraction	M-cells		F-cells	
	Control	Allopurinol	Control	Allopurinol
[^{14}C]adenine				
Uptake (dis./min/μg protein)	3134.9 ± 102.1	2794.8 ± 88.1†	2452.9 ± 98.4	3227.6 ± 120.3†
(total uptake, dis./min × 10^{-4})	(45.7 ± 2.8)	(47.3 ± 3.7)	(100.1 ± 8.9)	(138.2 ± 13.1)
Release medium (%)	17.0 ± 2.0	18.0 ± 2.5	22.0 ± 5.1	17.0 ± 1.8
Intracellular (%)	83.0 ± 4.8	82.0 ± 1.8	78.0 ± 3.7	83.0 ± 4.8
H^+-soluble, cellular (%)	75.4 ± 4.6	73.9 ± 3.0	65.5 ± 5.5	67.3 ± 2.6
H^+-insoluble, cellular (%)	7.6 ± 0.9	8.1 ± 0.5	12.5 ± 1.3	15.7 ± 1.8
[^{14}C]hypoxanthine				
Uptake (dis./min/μg protein)	249.8 ± 19.3	199.5 ± 16.9‡	185.4 ± 16.8	228.4 ± 16.9‡
(total uptake, dis./min × 10^{-3})	(40.4 ± 2.9)	(33.1 ± 2.7)	(76.6 ± 4.6)	(94.9 ± 6.0)
Release medium(%)	55.0 ± 3.5	37.0 ± 3.2†	34.0 ± 4.1	18.0 ± 1.4†
Intracellular (%)	45.0 ± 4.3	63.0 ± 3.6†	66.0 ± 5.5	82.0 ± 5.5‡
H^+-soluble, cellular (%)	36.2 ± 4.3	49.1 ± 5.4†	39.9 ± 4.6	58.9 ± 5.8†
H^+-insoluble, cellular (%)	8.8 ± 1.7	13.9 ± 1.2‡	26.1 ± 3.9	23.1 ± 4.5

* Cells were labeled in the presence and absence of 0.5 mM allopurinol (see Methods). Uptake was calculated by dividing the total uptake, as determined by the difference in the medium before and after incubation for 90 min, by the total cellular protein (dis./min.μg of protein). The results are given as the mean ± S. D. of four separate experiments. The distribution of label in the subsequent post labeling period of incubation was determined by measuring the activity in the release medium and in the H^+-soluble fractions. The activity in the intracellular and H^+-insoluble fractions was calculated by difference. These results are expressed as the mean percentage ± S. D. in the four experiments.

† Significant difference (t-test) from the control, $P < 0.01$.

‡ Significant difference (t-test) from the control, $P < 0.05$.

per cent in the mixed cell preparation to 90 per cent, or better, of cells beating either synchronously when in contact, or spontaneously, at a rate of 72–80 beats/min at room temperature.

Metabolic experiments were initiated on day 3 of culture by the addition of fresh media containing 1 μCi/ml of either [8-^{14}C]-adenine (40 mCi/m-mole) or [8-^{14}C]hypoxanthine (40 mCi/m-mole) to twenty replicate culture plates. The media used in the hypoxanthine experiments were modified by the omission of added hypoxanthine, a constituent of the nutrient medium [4]. The cultures were then incubated for a period of 90 min to label the cells. Ten cultures were exposed to allopurinol (0.5 mM) in the medium for 60 min prior to the initiation of the experiment and during the 90 min uptake period. The cells were then washed twice with unlabeled medium and incubated for an additional 120 min. Replicate medium and washings from the uptake, and from the incubation (release), periods were combined and immediately deproteinized with cold trichloroacetic acid (TCA) at a final concentration of 5%. The cells attached to each replicate plate were combined after scraping and rinsing them from the culture plates with cold 5% TCA, and then homogenized with a motor driven Potter–Elvehjem homogenizer. The acidified fractions were cleared by centrifugation in the cold. The acid-soluble and acid-insoluble fractions were stored overnight at − 20°.

Labeled purines were adsorbed from the acid extracts

Table 2. Distribution of acid-soluble purines in heart cell cultures 120 min after labeling with [^{14}C]adenine*

Fraction	M-cells		F-cells	
	Control	Allopurinol	Control	Allopurinol
Release medium				
Guanine	1.5 ± 0.7	0.6 ± 0.5	Trace	0.0
Xanthine	0.5 ± 0.7	2.2 ± 1.7	1.1 ± 0.4	0.9 ± 0.2
Inosine	72.7 ± 2.9	71.8 ± 2.8	58.9 ± 3.5	58.8 ± 3.7
Hypoxanthine	23.8 ± 2.4	24.2 ± 2.7	39.6 ± 3.2	39.8 ± 2.8
Adenosine	1.5 ± 0.3	1.3 ± 0.2	0.4 ± 0.2	0.5 ± 0.2
H^+-soluble intracellular fraction				
ADP+ATP	73.9 ± 3.8	82.5 ± 4.1†	72.0 ± 2.3	78.0 ± 1.9†
GMP	2.7 ± 0.5	2.0 ± 0.4	5.6 ± 1.5	1.9 ± 0.9†
IMP	2.3 ± 0.5	2.4 ± 0.5	1.4 ± 0.5	4.1 ± 1.4†
AMP	20.6 ± 2.3	12.0 ± 2.5‡	20.6 ± 2.4	14.7 ± 1.8‡
Adenosine	0.4 ± 0.3	0.7 ± 0.5	0.5 ± 0.4	0.7 ± 0.4
Inosine	Trace	Trace	0.0	0.7 ± 0.6
Hypoxanthine	0.0	0.4 ± 0.3	Trace	0.0

* Results are expressed as the mean percentage ± S. D. of the amount of label in the release medium and the acid-soluble intracellular fraction in four experiments (Table 1) after chromatographic separation and elution from cellulose or PEI-cellulose. ADP+ATP plus other pyrophosphates remain at the origin after two-dimensional chromatography on PEI-cellulose and were assayed together.

† Significantly different from control values, $P < 0.05$.

‡ Significantly different from control values, $P < 0.01$.

with norit, which was then washed with water and eluted with a mixture of ethanol (50%), pyridine (3%) and NH_4OH (1%) in water. Recovery of the labeled compounds after norit elution was between 85 and 95 per cent. The fractions were analyzed with appropriate purine markers on thin-layer cellulose (Baker) in the solvent *n*-butanol–methanol–H_2O–NH_4OH (60:30:30:1, v/v) and on PEI cellulose (Baker) with acetic acid (1 N, to 4 cm) and water in the first dimension, and 0.3 M LiCl in the second dimension. Before use, the PEI cellulose sheets were washed alternatively with distilled water, 10% NaCl and distilled water, dried, and then given a final wash chromatographically with distilled water.

The air-dried sheets were stored at 2° until used. After the chromatograms were developed, the ultraviolet absorbing areas were scraped into scintillation vials for counting. All samples to be counted were combined with 0.5 ml of a tissue solubilizer (Soluene, Packard Instrument Co., Downer's Grove, IL.) for 30-min before the addition of Bray's scintillation solution. Counting was done to a 95 per cent, or better, confidence level, and the results were corrected for quenching by the channels ratio method.

Results. The uptake of adenine was about thirteen times greater than the uptake of hypoxanthine in both M-cells and F-cells (Table 1). Most of the label from adenine was found in the acid-soluble intracellular fractions at the conclusion of the 120 min postlabeling period. In contrast, the hypoxanthine-labeled cells released a considerable proportion of intracellular label into the medium, especially in the M-cell cultures. In addition, the distribution of label indicated that the polynucleotide (H^+-insoluble) fraction of the F-cells had a greater percentage of label than did the same fraction of the M-cells after incubation with either adenine or hypoxanthine.

In the presence of allopurinol, purine uptake was enhanced by 23–31 per cent in the F-cells and was reduced by 11–20 per cent in the M-cells (Table 1). On subsequent incubation, a considerable proportion of the hypoxanthine label was now retained in the intracellular fractions of both cells in contrast to the control cells. An increase in retention did not occur in either cell culture after labeling with adenine.

The composition of labeled metabolites was determined after chromatographic separation of the purines in the release medium and in the acid-soluble intracellular fraction of the cultures labeled with adenine at the conclusion of the 120-min incubation period. A similar analysis of hypoxanthine-labeled cultures was not done owing to the limited amount of sample available. As shown in Table 2, the distribution of purine bases and nucleosides released into the medium of the adenine-labeled culture indicated that inosine was the prevalent nucleoside and that allopurinol did not alter the ratio of inosine to hypoxanthine found in the medium of either cell culture. The purines listed accounted for all of the label present in the release medium. The presence of labeled adenosine in the medium was confirmed by an additional chromatographic system utilizing buffered Whatman No. 1 filter paper, as described by Nestle and Roberts [6]. Purine nucleotides were not found in the medium at any time, as has been reported for cultured embryonic cardiac cells [7]. The appearance of labeled hypoxanthine in the medium of the M-cell culture is of interest because nucleoside phosphorylase activity has not been detected in myocardial cells of the guinea pig myocardium [1]. This may be a reflection of culture contaminants or of metabolic variation. The release of radioactive material into the medium of either cell culture did not change significantly after exposure to allopurinol. The amount of adenosine found in the medium after the incubation represents a minimal amount owing to the presence of adenosine deaminase, and possibly of nucleoside phosphorylase, activity in the calf serum used in the medium. The presence of these enzymes may equilibrate the released nucleosides and lead to a relatively uniform distribution of adenosine, inosine and hypoxanthine. However, the medium contained about three times as much adenosine after incubation with M-cells than after incubation with F-cells, and the ratio of adenosine, inosine and hypoxanthine in the medium was not the same after incubation with F-cells and M-cells. This suggests that each cell type releases varying amounts of these compounds. The finding of higher amounts of adenosine in the medium after incubation of M-cells supports previous studies implicating the release of adenosine as part of a local mechanism for vasodilation [1].

The composition of the labeled purine pool in the acid-soluble intra-cellular fraction is shown in Table 2. The adenine nucleotides predominate in both cell types, with an ADP + ATP/AMP ratio of about 3.5. Exposure to allopurinol altered the distribution of nucleotides by increasing the proportion of ADP + ATP in the intracellular pool, so that the above ratio rose to 6.9 and 5.3 in the M-cells and F-cells respectively. The increased ratio in allopurinol-treated cells might be expected to result in a decreased release of adenosine, but this does not appear to have been the case. In both cell types, the decrease in AMP was approximately proportional to the increase in ADP + ATP. In addition, the accumulation of IMP in F-cells that had been exposed to allopurinol suggests a block in the formation of GMP, since the relative amounts of IMP and GMP are essentially reversed from those found in the control cells.

DISCUSSION

Metabolic studies with labeled adenine and hypoxanthine were performed on cultured F-cells and beating M-cells segregated from the same heart tissue preparation. In both F-cell and M-cell cultures, it was found that the uptake of adenine was quantitatively greater than the uptake of hypoxanthine, and that the adenine-labeled cells released proportionally less of the intracellular label than did the hypoxanthine-labeled cells. The uptake of labeled hypoxanthine could be affected by dilution if an unlabeled compound was present in the medium. Since hypoxanthine was not added as a component of the nutrient medium when labeled hypoxanthine was included, it is possible that the calf serum contributed an amount sufficient to affect the observations. However, an analysis of the serum indicated that the amount of hypoxanthine present would add only 13 per cent to the total when labeled hypoxanthine was added. Therefore, it is unlikely that the lesser uptake of labeled hypoxanthine observed can be attributed to dilution. The availability of O_2 does not seem to account for the differential release data, since a rapid loss of incorporated purines from either source would have been expected if a limiting O_2 tension prevailed [8]. The low level of GMP found in the F-cells and M-cells corresponds to similar findings made with cultured lymphocytes and tumor cells in which the major pathway of guanine nucleotide formation from AMP appeared to be directly through IMP rather than through the nucleoside-hypoxanthine salvage route [9].

The observation that both F-cells and M-cells respond to allopurinol, or a metabolite of allopurinol, by increasing the ADP+ATP levels concomitant to a proportional decrease in AMP supports previous work on the myocardium. The allopurinol-treated myocardium, after a period of ischemia and hypoxia, developed an increased contractility and output, for which the restoration of high levels of ATP are apparently necessary [2, 3, 10, 11]. Assuming isotopic equilibration with cellular constituents, the intracellular purine pool in the cultured heart cells consisted primarily of nucleotides. The sum of ADP and ATP, taken as the percentage (Table 2), would then represent an approximation of the energy charge [12], and would indicate that the cultured cells had an energy charge of about 0.72.

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