times greater than that of cows. In milk of strain C57BL/6J, the lipid concentration was 41.6 ± 7.3%, and butterfat was 22.0 ± 3.7%(2).

*Summary.* Fatty acid composition of milk from DBA/2J, C57BL/6J, B6D2F$_1$ and 3 backcross types classified according to coat color (B6D2F$_1$ × DBA/2J) was obtained by gas liquid chromatography. The total of fatty acids in mouse milk ranged from 7.79 to 19.02 g/100 ml and 14 fatty acids were identified. Measureable quantities, 0.02 g/100 ml or more, of $C^{10}$ to $C^{22:1}$ were present. Eight were saturated fatty acids and of the 6 unsaturated ones, 4 were singly unsaturated and 2 were doubly unsaturated. Linoleic, palmitic, and oleic acids occurred in highest concentration, probably because the dietary corn oil is especially rich in these acids. Linolenic acid and arachidonic acids were absent in milk, and are also lacking in corn oil. Although genetic analysis of fatty acid composition could not be done because of procedural difficulties in obtaining sufficiently large samples of milk from individual mice, the possibility of genetic influences upon fatty acid levels exists for reasons which are discussed.

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## Cytosine Arabinoside Sensitivity in Actinobolin-Resistant *Streptococcus faecalis*: The Basis of a Utilitarian Microbiological Assay.* (31811)

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Cytosine arabinoside (1-β-D-arabinofuranosylcytosine hydrochloride) has been reported to inhibit a variety of experimental mammalian neoplasms *in vivo*(1-5). It also has antiviral activity: inhibition in cell culture of the DNA viruses of herpes simplex, B-virus, pseudorabies, vaccinia, swine pox and fowl pox, but lack of inhibition of several adenoviruses and RNA viruses has been reported(6-12). Cytosine arabinoside has been briefly reported to inhibit the anaerobic growth of *Escherichia coli*(13) but a definitive evaluation of the antimicrobial properties of this compound has not been published.

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Initial reports indicate that cytosine arabinoside has antileukemic activity in humans (14-20). The applicability of first order reaction kinetics of cell kill in leukemia and other tumors by antineoplastic agents(21) has emphasized the need to determine inhibitor concentration-time relationships in both experimental animals and humans. A microbiological assay for cytosine arabinoside has been developed and its utility demonstrated for biological samples.

*Antimicrobial activity determinations.* In a search for a potential assay microorganism, 325 microorganisms (bacteria, yeast and fungi) were tested for sentitivity to cytosine arabinoside using previously described methods(22) (see reference 23 for complete listing of the cultures used). Agar-diffusion

TABLE I. Collateral Sensitivity of Agent-Resistant Strains of Certain Bacteria to Cytosine Arabinoside.

| Bacteria | Diameter of zones of inhibition (cm)* | | |
|---|---|---|---|
| | Exp 1 | Exp 2 | Exp 3 |
| *Streptococcus faecalis* ATCC 8043 | 0 | 0 | 0 |
| *Streptococcus faecalis* ATCC 8043-resistant to actinobolin | 4.2 | 3.8 | 3.8 |
| *Escherichia coli* ATCC 9637 | 0 | 0 | 0 |
| *Escherichia coli* ATCC 9637-resistant to 6-diazo-5-oxo-L-norleucine | 1.5 | 1.6 | 1.6 |
| *Escherichia-coli* B (ORNL) | 0 | 0 | 0 |
| *Escherichia coli* B/r | 1.7 | 1.7 | 1.7 |

* Surrounding filter paper-discs (diameter 1.27 cm) impregnated with 100 μg cytosine arabinoside and placed on the surface of seeded agar plates.

methods were employed(24). The spectrum of microorganisms tested for sensitivity included representatives of (a) 20 genera of Gram-positive, Gram-negative, and acid-fast bacteria and derived inhibitor-resistant strains, (b) 28 genera of fungi (filamentous and non-filamentous, and (c) 11 genera of algae. Cytosine arabinoside was found to be essentially devoid of significant antimicrobial activity under these test conditions. At a maximum concentration of cytosine arabinoside tested (100 μg/disc) only a few bacteria were found to be inhibited. However, a strain of *Streptococcus faecalis* ATCC 8043 resistant to the antibiotic actinobolin(25) was sufficiently sensitive to cytosine arabinoside to consider it as a microbiological assay organism. Other microorganisms found to be sensitive to cytosine arabinoside under these test conditions were a strain of *Escherichia coli* ATCC 9637 resistant to 6-diazo-5-oxo-L-norleucine(22) and the radiation-resistant strain of *E. coli* designated *E. coli* B/r(26) (see Table I).

*Microbiological assay for determination of cytosine arabinoside (NSC 63878) in mouse blood.* A strain of *Streptococcus faecalis* ATCC 8043, >50-fold resistant to actinobolin and designated *Strep. faecalis*/ACB, was maintained on Folic Acid Assay Agar (Difco Laboratories, Detroit, Mich.) slants containing 1 mg actinobolin/ml of medium. This medium was used exclusively in this assay. For inocula, broth cultures of *Strep. faecalis*/ACB were grown for 18 hours at 37°C in the medium described above supplemented with 1 mg actinobolin/ml. The cells were washed by replicate centrifugation and resuspended in 0.85% NaCl solution. The suspension was adjusted to 0.7 optical density units in a Bausch and Lomb "Spectronic 20" colorimeter at 660 mμ. Such a suspension contains about $1 \times 10^8$ viable cells/ml. This suspension was diluted 1:20 in 0.85% NaCl solution and 10 ml of the diluted cell suspension was added to one liter of agar medium (50-55°C). Five ml of the seeded medium was added to petri plates containing 8.0 ml solidified, sterile agar medium.

A stock solution of cytosine arabinoside was prepared in sterile saline (0.85% NaCl solution). This was appropriately diluted so that when 0.08 ml of the various dilutions was added to filter paper discs, the following concentrations were obtained (μg cytosine arabinoside/disc): 60, 40, 20, 10, 8, 6, 4, 2, 1, 0.8, 0.6, 0.4, 0.2, and 0.1.

An additional cytosine arabinoside stock solution was prepared in which the drug was dissolved in mouse blood and diluted with saline. A third stock solution was prepared but in which cytosine arabinoside was dissolved in and diluted with modified Eagle's medium(27). Filter paper discs (1.27 cm diameter, No. 740-E, Schleicher and Schuell Co., Keene, N. H.) were impregnated with 0.08 ml of the various solutions, containing graded concentrations of drug. These discs were placed on the surface of each seeded agar plate and pressed down securely with flamed forceps. All plates and drug concentrations/disc were prepared in triplicate. Each individual plate contained a maximum of 3 discs: 2 discs contained individually either experimental samples or standard curve solutions of different concentrations. A third control disc, containing an empirically selected concentration (0.2 μg cytosine arabinoside/disc) of drug, was added to each plate to allow for correction of plate-to-plate variation in zone sizes. All plates were incubated at 37°C

for 18-20 hours simultaneously with plates which contained discs impregnated with blood from animals which received cytosine arabinoside. The resulting zones of inhibition on the triplicate standard plates were measured and corrected as follows: If the mean diameter of all the control disc zones was greater than that of an individual control disc zone, the difference was added to all of the zones on that plate. Conversely, if the average diameter of the control disc zones was less than that of an individual control disc, the difference was subtracted from all the zones on that plate. The mean diameter of these corrected zones was determined for each drug concentration and entered on semilogarithmic paper—the diameter of zones on the arithmetic scale and concentrations of drug/disc on the logarithmic scales. A straight line of visual best fit was drawn through the points thus obtained. This line constituted a standard curve. From the zones which developed around the discs which received blood samples from the treated animals, drug concentrations ($\mu$g/disc) were read directly from the abscissa of the standard curve. Drug concentrations (mg/ml) in the blood of treated mice were calculated by multiplying the $\mu$g/ml values by the appropriate dilution factor. Standard curves were prepared each time blood samples were assayed for drug levels. Fig. 1 illustrates the standard curve obtained when cytosine arabinoside is dissolved in 0.85% NaCl. Essentially identical results were obtained when cytosine arabinoside was dissolved in either heparinized whole mouse blood or in a modified Eagle's medium(27).

*Determinations by microbiological assay of cytosine arabinoside (or cytotoxic equivalents) in mouse blood following various routes of administration.* In all experiments $BDF_1$ mice (mixed sexes, 18-22 g) were used. Drug was administered at various doses in 0.2 ml of 0.85% NaCl (with the exception of experiments involving the constant perfusion machines). Three methods of securing blood samples from treated animals were used: (a) repeated cardiac puncture in anesthetized mice; (b) replicate entry into the postorbital sinus; and (c) visual cardiac puncture of the exposed heart immediately after sacrifice. Our total experience is summarized in Table II.

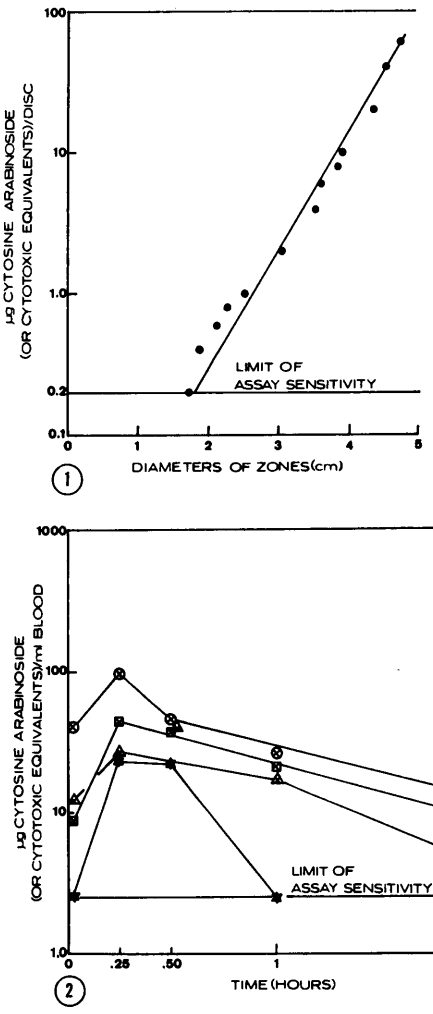


FIG. 1. Standard curve, cytosine arabinoside dissolved in 0.85% NaCl. Assay organism: *Streptococcus faecalis* ATCC 8043/ACB.

FIG. 2. Mouse blood levels following single dose (IP) of cytosine arabinoside. Mice bled by visual cardiac puncture at indicated times. Each point represents the mean of observed values from 8 mice. Assay organism: *Streptococcus faecalis* ATCC 8043/ACB. Circle x—250 mg/kg, square x—150 mg/kg, triangle x—100 mg/kg, star—50 mg/kg.

Fig. 2 illustrates graphically the levels of drug detected in the blood of mice which were given 250, 150, 100 or 50 mg/kg doses of cytosine arabinoside by intraperitoneal injection. Blood samples were collected by

TABLE II. Drug Levels in Blood of Mice Treated with Cytosine Arabinoside (NSC 63878).

| Route of administration | Dose | Maximum blood levels and time detected | Method of securing blood samples |
|---|---|---|---|
| IP | 250 mg/kg | 100 μg/ml—10 min | Cardiac puncture |
| | 150 " | 45 " —10 " | " " |
| | 100 " | 27 " —10 " | " " |
| | 50 " | 23 " —10 " | " " |
| IP | 100 " | 75 " —10 " | Postorbital sinus |
| | 50 " | 37 " —10 " | " " |
| IV | 100 " | 120 " — 2 " * | Cardiac puncture |
| | 50 " | 60 " — 2 " * | " " |
| IV | 100 " | 380 " — 2 " * | Postorbital sinus |
| | 50 " | 190 " — 2 " * | " " |
| PO | No drug detected | | |
| CP† | 1.66 mg/kg/min‡ | 35 " —60 " | " " |
| | .83 " " § | 20 " —60 " | " " |

* First sampling period.
† Constant perfusion (intraperitoneal).
‡ and § After 1 hour, these rates of drug infusion are equivalent to a single dose (100 or 50 mg/kg, respectively) of cytosine arabinoside.

visual cardiac puncture. Highest drug concentrations were found approximately 10 minutes post-drug administration regardless of the amount of drug given to the animals. Measurable quantities of cytosine arabinoside were present 2 hours after administration of drug in those animals which received the 3 highest doses of drug.

*Discussion.* An explanation for the singular sensitivity of the actinobolin-resistant strain of *Strep. faecalis* to cytosine arabinoside is lacking. Actinobolin is known to be an inhibitor of protein synthesis in both bacteria(28) and in mammalian cells in culture(29), but nothing has been reported regarding the mechanism(s) of resistance to this antibiotic. Actinobolin-resistant cultures of *Bacillus subtilis* ATCC 6051, *Escherichia coli* ATCC 9637, or *E. coli* ATCC 11303 were all insensitive to inhibition by cytosine arabinoside.

Regardless of the biochemical explanation for the sensitivity of *Strep. faecalis*/ACB to cytosine arabinoside, a sensitive and precise assay has been developed which is suitable for estimating cytosine arabinoside (or cytotoxic equivalents) in mouse blood.

*Summary.* The observation that cytosine arabinoside was inhibitory for a strain of *Streptococcus faecalis* resistant to the antibiotic actinobolin served as the basis for the development of a microbiological assay for cytosine arabinoside in biological materials. Small volumes of less than one ml are sufficient for the assay and as little as 3 μg of cytosine arabinoside/ml of sample can be measured. The biological utility of this assay has been demonstrated.

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## Lactate Dehydrogenase Activity in Dog Adipose Tissue.* (31812)

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In the course of our studies in fatty acid metabolism it became desirable to investigate the enzyme systems responsible for channeling metabolites to alternate metabolic pathways. An approach of this sort can provide information that is not necessarily obtained by following turnover rates of specific metabolites, the synthesis and degradation of important intermediates or by assaying end products of various routes.

Some 30 years ago fat stores were believed to be metabolically entirely passive. Experimental evidence of the past few years (1-4) has changed this concept radically, revealing adipose tissue as the site of very active metabolic processes of great diversity.

Lactic dehydrogenase of dog adipose tissue was one of the enzymes whose activity was of interest to us. Although lactate dehydrogenase catalyzes the reversible oxidation of lactate irrespective of its origin, each tissue enzyme possesses characteristic physicochemical properties. Therefore, it was important first to provide some of the necessary chemical and physiological background for subsequent interpretation of metabolic data in a meaningful manner.

This paper is concerned with some of the functional and kinetic characteristics of dog adipose tissue lactate dehydrogenase (LDH).

*Materials and methods.* Subcutaneous inguinal and pericardial adipose tissue were used as the source of enzyme. A 10% homogenate in isotonic KCl solution was prepared in a ground glass close-fitting motor driven homogenizer. The homogenate was centrifuged for 30 minutes at a speed of 100,000 $\times$ *g* in a Spinco preparative ultra-centrifuge. The supernatant fluid was diluted 240-fold as a rule for an activity level suitable for measurement.

Measurement of enzyme activity was usually made in 0.1 M phosphate buffer at physiological pH (7.4) at $5.0 \times 10^{-4}$ M pyruvate concentration in the presence of $1.3 \times 10^{-4}$ M final concentration of DPNH. Decrease of optical density at 340 m$\mu$ due

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