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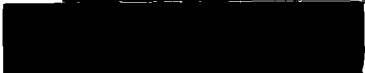
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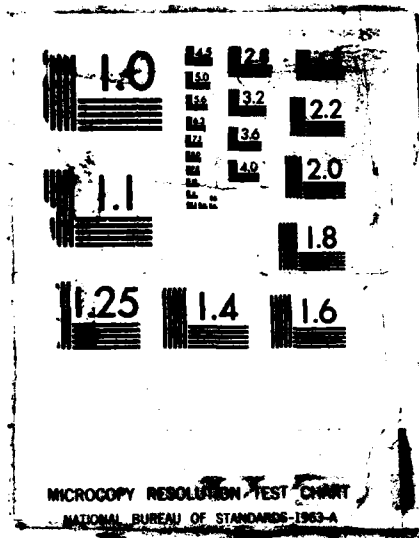
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CERCARIAL PENETRATION STUDIES: STEPS TOWARD CHEMOPROPHYLAXIS IN  
SCHISTOSOMIASIS

ANNUAL REPORT

B. SALAFSKY AND A.C. FUSCO

OCTOBER 15, 1986

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND  
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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)  This report summarizes the research we have conducted during the first year of contract #DAMD17-85-C-5180. The scope of this research centers on elucidating the biochemical mechanisms involved in cercarial (Schistosoma mansoni) skin penetration and the evaluation of eicosanoid inhibitors as possible prophylactic agents. The most significant findings to date are: 1) Cercarial eicosanoid production is pH dependent. 2) Esculetin was effective <u>in vitro</u> at inhibiting cercarial penetration of an agar:gelatin substrate. However, the drug was not effective <u>in vivo</u> , probably due to its short serum half-life.		

3) We have tentatively identified cercarial LTB<sub>4</sub> (or metabolites) production as a correlate of cercarial penetration. 4) After examining various in vitro methods used to "transform" cercariae into schistosomules, we could find no significant correlations between ultrastructural and loss of water tolerance or eicosanoid production. This is particularly significant since ultrastructural changes have been the predominate method used to evaluate transformation methods. 5) Cercariae more readily penetrate the skin of SENCAR mice, a strain with reportedly high skin lipoxigenase products, than they do the skin of either ICR or NMRI strains. This provides indirect evidence of the importance of lipoxigenase products in cercarial penetration. *Keywords:*

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### SUMMARY

This report summarizes the research we have conducted during the first year (14 August 1985 to 14 August 1986) of contract #DAMD17-85-C-5180. The scope of this research centers on elucidating the biochemical mechanisms involved in cercarial (*Schistosoma mansoni*) skin penetration and the evaluation of eicosanoid inhibitors as possible prophylactic drugs. During the past year, nine areas of research were pursued:

1. The Effect of Low Linoleate Concentration on Cercarial Penetration and Transformation. We have concluded that while cercarial penetration and transformation rates can increase over time when exposed to very low levels of linoleate (0.003 to 0.03mM), this increase cannot be correlated to either eicosanoid production or phospholipid levels. Since cercarial eicosanoid levels do correlate with increasing concentrations of moderate levels linoleate (0.03 > 9mM), we have concluded that using low linoleate concentrations to help elucidate the biochemistry of cercarial skin penetration is not a fruitful approach.

2. Role of pH in Cercarial Eicosanoid Production. We have found that pH directly affects cercarial eicosanoid production when agar is utilized as a penetration substrate. Cercarial eicosanoid production is increased at slightly acidic pH when compared to slightly alkaline pH.

3. The Effect of Ibuprofen and Esculetin on Cercarial Penetration, Transformation and Eicosanoid Production (in vitro). Ibuprofen has no effect on cercarial penetration or transformation rates at concentrations as high as 10mM when it is incorporated into a gelatin:agar matrix. Esculetin (1mM) was effective at inhibiting cercarial penetration, transformation, and eicosanoid production, however its ability to inhibit either process was pH dependent.

4. Validation of the [75Se] Labelling Technique for Tracking Cercarial Penetration and Transformation in vivo. We have validated the technique of using [75Se] labelled cercariae for the in vivo measurement of cercarial penetration and migration in ICR mice.

5. The Effect of Esculetin on Cercarial Penetration and Transformation in vivo. Esculetin concentrations of 100mg/kg or 200mg/kg fails to protect mice when challenged with cercariae via the tail route; most probably due to its short serum half-life. Thus, esculetin is no longer being investigated as a possible prophylactic agent.

6. The Biochemical Mechanisms Involved in Cercarial Transformation. A series of experiments (still in progress) were conducted examining various cercarial transformations methods with respect to cercarial ultrastructure (EM), loss of water tolerance and eicosanoid production. Currently there are no correlations between ultrastructure and biochemical parameters. Possibly ultrastructural changes alone are not good indicators of cercarial transformation. We are in the process of extending the scope of these studies to include RNA, DNA and protein synthesis.

7. The Role of Skin Eicosanoid Production in Cercarial Penetration. Cercariae have significantly different penetration rates when exposed to mouse strains (ICR, NMRI, SENCAR) with reportedly different skin eicosanoid levels. We are in the process of confirming skin eicosanoid production in each strain as well as extending the scope of this study to include other mouse strains.

8. Primary Drug Screening of Various Eicosanoid Inhibitors. Both caffeic acid and ketoconazole (known eicosanoid inhibitors) were tested for their ability to inhibit cercarial stimulation by linoleate. Of the two, ketoconazole

zole was the most effective drug. We are currently extending these studies to include other eicosanoid inhibitors.

9. Preliminary Experiments on the Use of an Artificial Skin Membrane to Investigate Cercarial Penetration and Transformation Mechanisms in vitro. Thus far, cercariae have not been able to penetrate an artificial skin membrane composed of keratin:chitin or keratin:collagen. These studies are still continuing.

**FOREWORD**

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

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### STATEMENT OF PROBLEM

Schistosomiasis is a parasitic, debilitating disease found in tropical and semi-tropical areas of the world. Many of these areas are of potential military interest (i.e. South America, North, Central, West Africa and the Near East) due to unstable governments, vital raw materials, and incursion by powers unfriendly to the United States. There currently does not exist an effective prophylaxis for schistosomiasis. The combat soldier, and possibly support personnel serving in these regions, is therefore at risk of infection. The studies in progress for this contract afford an opportunity to more fully understand the biochemical mechanisms involved in cercarial (see glossary) skin penetration and transformation; the first step in infection. With basic research, we believe it will be possible to rationally develop a novel approach to chemoprophylaxis in schistosomiasis. This contract centers on cercarial stimulation by essential fatty acids and resultant eicosanoid production as a possible specific target of chemoprophylactic attack.

### THE BACKGROUND

We have demonstrated that cercarial eicosanoids (lipoygenase and cyclooxygenase products) produced as the result of stimulation by skin surface essential fatty acids (EFA) are important mediators for successful cercarial penetration and transformation. These findings have been supported by: 1) the stimulatory nature of EFA and their role in eicosanoid production, 2) the reduction in penetration response and transformation when cercariae are treated with eicosanoid inhibitors, 3) the wide variety of eicosanoids produced when cercariae are stimulated by EFA, 4) the ability of eicosanoid inhibitors to dramatically decrease cercarial eicosanoid production, 5) the dose-response nature of eicosanoid stimulation by EFA, 6) the correlation of cercarial penetration with lipoygenase products (i.e. LTs and HETEs) and 7) the identification of  $LTB_4$  or metabolites as correlates of the penetration process. Given these data we have formulated an "eicosanoid hypothesis" that states: (1) Cercariae are stimulated to penetrate skin via skin surface essential fatty acids. (2) The fatty acids are incorporated by cercariae and serve to trigger cercarial eicosanoid production. (3) These eicosanoids either act directly within cercariae to play a vital role in the biochemical events associated with transformation and/or are secreted by cercariae into the host, resulting in immuno-modulatory influences that favor penetration.

### APPROACH TO THE PROBLEM

Given the Statement of Problem and Background as detailed above, this contract seeks to approach the problem of chemoprophylaxis in schistosomiasis via:

a) Conducting preliminary investigations on the in vivo prophylactic efficacy of ibuprofen, esculetin and related eicosanoid inhibitors.

b) Studying the role of skin essential fatty acids, other skin lipid components and cercarial eicosanoid production in relation to the cercarial penetration and/or transformation responses and drug intervention.

c) Study the mechanisms of action of ibuprofen, esculetin, and related eicosanoid inhibitors at the level of cercarial receptor sites for eicosanoids and essential fatty acids.

#### **NARRATIVE SUMMARY OF RESEARCH PERFORMED FROM 14 AUGUST 1985 - 14 AUGUST 1986.**

Given the Approach to the Problem as defined above and in our contract the following nine areas of research were conducted from 14 August 1985 to 14 August 1986.

##### **1. The Effect of Low Linoleate Concentration of Cercarial Penetration and Transformation.**

Initially, we determined that cercariae exhibit a dose-response phenomena with respect to transformation, penetration and eicosanoid production when exposed to varying concentrations of linoleate for 1 hr at 37°C. Low linoleate concentrations (i.e. 0.003 and 0.03mM), resulted in minimal penetration, transformation, and eicosanoid synthesis rates. Increasing the linoleate concentration from 0.003 to 9mM resulted in an increase in penetration rates, LT and HETE levels until 1mM linoleate is reached when transformation rates and PG production also begin to increase. At 9mM a toxic concentration of linoleate was reached. Since these experiments were conducted by exposing cercariae to linoleate for 1 hr, we wondered whether cercariae would respond to low linoleic acid levels (0.003 & 0.03mM) if the time of exposure was increased. Thus we measured cercarial penetration, transformation and eicosanoid production after 1, 2, and 4 hrs exposure to either 0.003 or 0.03mM linoleate. Our data has shown that at 0.003mM linoleate, the percentage of cercariae penetrating an agar:gelatin matrix gradually increased from 0% at 1 hr to a high of 70% after 4 hrs at 37°C; however, transformation rates did not change. Control plates containing no linoleate did not show an increase in penetration rates. At 0.03mM linoleate, the percentage of cercariae penetrating an agar:gelatin matrix increased from 25 to 55% between 1 and 4 hrs, while transformation rates increased from 20 to 40% over the same time period. Thus it appears that cercariae can respond to low linoleate concentrations over time, however the time periods involved suggest that this is not a physiological response (cercariae can normally penetrate skin within 5 mins). This interpretation is also supported by HPLC analysis of cercarial eicosanoid and phospholipid production in the above experiments. Neither phospholipid or eicosanoid levels were significantly correlated to cercarial penetration or transformation. In fact, eicosanoid and phospholipid synthesis were minimal. However, all the experiments reported above were conducted between pH 7.2 and 7.5 and we have since found eicosanoid production to be pH dependent, favoring a slightly acidic pH (see #2 below). Therefore, the possibility exists that different results may be obtained using a lower pH and/or RIA assay instead of HPLC as a measure of eicosanoid production (RIA is more sensitive). However, we currently feel that continuing this line of research will not enable us to examine the early biochemical steps involved in cercarial skin penetration, as we had hoped. Hence this line of research has been halted.

##### **2. Role of pH in Cercarial Eicosanoid Production.**

We have noted that the cercarial transformation process is pH sensitive. Cercarial penetration through an agar:gelatin matrix containing 3mM linoleate

was 90-100% complete over a pH range of 5.5 to 8.0. However, transformation of cercariae occurred optimally between pH 6.2 and 7.3. Measurement of overall eicosanoid production showed that eicosanoid synthesis was greater at pH 6.55 than 7.2. These experiments demonstrate the importance of maintaining accurate pH levels in all experiments involving cercarial penetration and transformation.

### 3. The Effect of Ibuprofen and Esculetin on Cercarial Penetration, Transformation and Eicosanoid Production (in vitro).

We also finished a series of experiments testing the effect of both ibuprofen and esculetin on cercarial penetration, transformation and eicosanoid production in vitro using our gelatin:agar plate methodology. Previously, these experiments were done in a liquid ELAC medium containing 3mM linoleate (37°C, 1 hr, 13000 cercariae) with varying concentrations of either ibuprofen or esculetin. We have found dramatic differences using the agar:gelatin substrate and otherwise identical conditions. For example, ibuprofen is not effective at inhibiting either penetration or transformation at concentrations as high as 10mM at a pH of 6.0 or 7.2 when incorporated into an agar:gelatin matrix, however ibuprofen is effective at much lower concentrations when cercariae are incubated in a liquid medium (ELAC).

Esculetin affects cercariae differently depending on the the pH of the agar:gelatin substrate. At pH 6.6 esculetin (1mM) inhibits penetration but at more alkaline pH levels it has no effect on penetration, rather it preferentially inhibits transformation. Cercarial transformation rates fell below control levels at pH values less than 6.4 and greater than 6.7 but remained normal in the range of 6.4 to 6.7. However, cercarial penetration decreased in this pH 6.4 to 6.7 range, but otherwise remained at control levels. When the effect of esculetin on cercarial eicosanoid was measured at pH 6.55 and pH 7.2, we noted an overall decrease in eicosanoid synthesis. However, at pH 7.2, two eicosanoids with retention times of 38'38" (Lt region) and 1:02 (HETE region) were found in increased amounts when compared to controls. This finding is particularly significant considering that cercariae exposed to esculetin at pH 7.2 have normal penetration while those exposed to esculetin at pH 6.55 have reduced penetration rates. Thus, we believe that we have identified two eicosanoids, one LT and one HETE, that are involved in the penetration process. In addition, we note that both cercarial transformation and eicosanoid production were pH dependent. A change of less than 1 pH unit can cause a shift in the species of eicosanoid produced as well as have dramatic effects on cercarial transformation. Further analysis revealed that LTB<sub>4</sub> and/or its metabolites are the leukotriene species involved in the penetration process. The results of these experiments have been submitted for publication to Molecular and Biochemical Parasitology. A copy of this manuscript has also been forwarded to SGRD-RMS.

### 4. Validation of the 75[Se] Labeling Technique for Tracking Cercarial Penetration and Transformation in vivo.

Since obtaining our [75Se] license we have run numerous studies evaluating the technique of using [75Se] labelled cercariae for the measurement of cercarial penetration and migration. In our hands, using the ICR mouse strain between 78.5 and 87.9% of labelled cercariae penetrate mouse tail skin. Of these 77.6% are found in the lungs at 7 days, and 18.5% remained in tail

skin (representing dark dots on the autoradiograph). These results compare favorably with previously published data; hence this technique has been validated in our laboratory.

#### 5. The Effect of Esculetin on Cercarial Penetration and Transformation in vivo.

We have finished evaluating a series of experiments in which the effect of various esculetin concentrations on cercarial penetration were studied in vivo in mice. The experiments were setup according to the following goals: a) to develop an effective esculetin HPLC assay that could be used to determine esculetin levels in mouse skin and blood, b) to determine normal (untreated) cercarial penetration and migration rates in mice using the [<sup>75</sup>Se] labeling technique, and c) to determine cercarial penetration and migration rates in mice using the [75Se] labeling technique given an esculetin dose regime.

a) Development of an HPLC technique for measuring esculetin levels in skin and plasma. The method we have developed for the extraction of esculetin from blood and skin allows us to recover approximately 56% of exogenously added esculetin. Plasma was assayed directly for esculetin after precipitating protein with 5% TCA (1:1 v/v). Tail skins were homogenized in 10 ml of 0.1N NaOH after which 10ml of 5% TCA was added. The homogenate was centrifuged at 6000 rpm for 10 min. The supernatant was removed and the pellet re-extracted. The two supernatants were combined, lyophilized, dissolved in 1ml distilled water, micro-centrifuged and analyzed via HPLC. Standard curves are prepared from plasma spiked with a known quantity of esculetin and 1:1 (v/v) of 5% TCA. The HPLC method utilized for detecting esculetin was a linear 20-80% methanol gradient over 20 minutes using optical detection at 330nm on a RP C-18 column. This method gave satisfactory results down to 50-100 ng esculetin. We have determined that 500mg/kg esculetin is lethal to 100% of mice injected IP. At 100mg/kg IP esculetin the following plasma and tail skin esculetin levels are obtained:

Time <sup>o</sup> (min)	Tail Skin (ug Total Content)	Blood Plasma (ug/ml)
15	7.38	39.4
30	11.82	97.0
90	-----	24.1
180	3.30	00.0

time post-injection of 100mg/kg esculetin

The data indicated that esculetin was rapidly excreted from the body. This can be seen visually by urine color (dark yellow/orange) during the first 60 min after esculetin injection. Peak plasma concentrations are only 97ug/ml plasma at 30 min post-injection, and tail levels are even lower. It is doubtful that a one time dose of esculetin can be an effective chemoprophylactic agent.

b) Tracking of [75Se] Labeled Cercariae in Untreated Mice. See #4 above.

c) The role of esculetin as a prophylactic inhibitor of cercarial penetration. Esculetin (100mg/kg) was injected IP into 18 mice. Five mice were

exposed to cercariae immediately after injection, five mice were exposed to cercariae 45 min after esculetin injection and the remaining mice were sacrificed at 15, 30, 90 and 180 min after esculetin injection to determine blood and tail skin esculetin levels. In addition 5 mice were given an IP injection of esculetin vehicle and exposed to cercariae. All mice were left exposed to cercariae via the tail route for 1 hr. The results were as follows:

Cercarial Exposure (min after esculetin injection)	%Cercariae Recovered (7 days post-exposure)
100mg/kg esculetin 0-60 min	tails $17.5 \pm 3.05$ lungs $59.2 \pm 8.99$
100mg/kg esculetin 45-105 min	tails $18.2 \pm 2.67$ lungs $69.3 \pm 2.69$
Controls	tails $18.5 \pm 5.58$ lungs $68.2 \pm 0.98$

A very slight reduction (10%) in the number of cercariae that migrated to the lungs 7 days post-exposure was noted only for those mice exposed immediately after a 100mg/kg IP dose of esculetin. Since the numbers of cercariae remaining in the tails after 7 days were the same among all 3 groups and esculetin is very short lived *in vivo*, we concluded that the cercarial reduction occurred during penetration. Blood levels of esculetin during the 1st hour after an IP dose of 100mg/kg reached a high of ~97ug/ml. The entire tail skin however has only ~12ug (probably in skin capillary beds). By comparison, our agar:gel penetration plates gave significant inhibition of cercarial penetration and transformation at 1mM esculetin, a concentration of ~174ug/ml. Thus, skin levels were 15x lower and blood levels just under 2x lower than esculetin concentrations that were effective *in vitro*. In addition, when mice were exposed to 200mg/kg esculetin IP using the above design no significant differences between experimental and control mice were found. Hence, we believe the slight reduction shown at 100mg/kg was not biologically significant. Several experiments were also undertaken using a time course of 100mg/kg and 50mg/kg esculetin given every 1 1/2 hr IP x 4. Due to the toxicity of the vehicle (esculetin is not soluble in saline) these experiments were inconclusive. Given the short half-life of esculetin (< 90 min) we do not believe that esculetin has any practical application as a chemoprophylactic agent, hence we are no longer investigating its effect of *in vivo*. We have decided to screen several other potential drugs that are either more potent lipoxygenase inhibitors or have more favorable pharmacokinetics.

#### 6. The Biochemical Mechanisms Involved in Cercarial Transformation.

We are just finishing a series of experiments investigating various cercarial transformation methods with respect to loss of water tolerance, tail loss, eicosanoid production, ultrastructure and RNA, DNA and protein synthesis. We have completed those studies involving loss of water tolerance, eicosanoid production (via RIA) and ultrastructure (via EM), but not those involving RNA, DNA and protein synthesis. Four transformation methods have been utilized in this study: chemical stimulation by 3mM Linoleate, mechanical shearing of tails, chemical/mechanical combination and incubation in buffer.

In addition, we have repeated these studies with both ELAC and Saline buffers. Cercariae were analyzed for water tolerance and eicosanoid production at 1, 2, 4 and 6 hrs, while ultrastructure was examined after 1 and 4 hrs of incubation. Water tolerance was assayed by the use of trypan blue (dead cercariae stain dark blue, whereas living cercariae are light blue to clear in color). The results were surprising. Overall, ELAC was a much better buffer than saline for inducing cercarial transformation as measured via loss of water tolerance. However, only those cercariae stimulated chemically lost their tolerance to fresh water after 6 hrs of incubation at 37C ( $97\% \pm 1.50$ , n=8). Mechanically transformed cercariae (ELAC) and those incubated in ELAC at 37C had highly variable transformation rates (=loss of water tolerance) reaching  $64.9\% \pm 13.48$  and  $58.1\% \pm 13.54$  after 6 hrs respectively. After 4 hours of incubation both mechanical and incubated cercariae had less than a 15% loss of water tolerance. Data for LTB<sub>4</sub>, PGE and 5-HETE production and EM ultrastructure are given in Table I. RNA, DNA and protein synthesis data are currently being collected. The results of our studies open questions on experimental results in much of the schistosomiasis literature, especially immunological data, collected utilizing mechanically transformed cercariae. In addition, these data show that ultrastructural changes may not be good indicators of cercarial transformation, since all transformation methods gave similar ultrastructural changes even though biochemical changes were vastly different.

#### 7. The Role of Skin Eicosanoid Production in Cercarial Penetration.

We have completed a series of experiments examining the role of skin eicosanoid production in relation to successful schistosome penetration and migration. Several strains of mice, that have been reported in the literature to have varying levels of skin eicosanoids, were used as models in these studies. Cercariae labelled with [75Se] were used to track skin penetration and migration in SENCAR, ICR and NMRI mouse strains. NMRI mice have been reported to have decreased levels of lipoxigenase products, SENCAR mice have been reported to have elevated levels of lipoxigenase products and ICR mice were used as normal controls. The following results were obtained:

Time	Mouse Strain		
	ICR	Sencar	NMRI
24hrs-Tails			
%Penetration	$79.72 \pm 5.03$	$100.06 \pm 3.17$	$68.70 \pm 4.79$
7days			
%In Tails	$9.44 \pm 1.14$	$11.89 \pm 1.53$	$26.57 \pm 3.31$
%In Lungs	$80.79 \pm 2.83$	$80.19 \pm 4.07$	$48.75 \pm 3.51$

N = 11 for ICR, 13 for Sencar, 5 for NMRI (the data for an additional 8 NMRI mice is currently being processed)

These results clearly show a difference in both penetration and migration rates between strains. Based on the reported differences in mouse strain eicosanoid production, a correlation between skin eicosanoid production and cercarial penetration can be made. We are in the process of confirming tail

skin eicosanoid production in each of these mouse strains to make sure such a correlation can be made. In addition, we have decided to add several mouse strains with known immunological deficiencies in order to extend the scope of this study. However, even if these penetration and migration rates can be correlated with skin eicosanoid production, it is important to realize that there may be other host genetic factors affecting schistosome infection. Future studies may establish relationships between these host factors, skin eicosanoids, and infection rates.

#### 8. Primary Drug Screening of Various Eicosanoid Inhibitors.

We have begun in vitro screening of several compounds that have been reported to be either potent lipoxigenase inhibitors or having some anti-schistosomule activity. Screening was done by measuring cercarial stimulation by linoleate. Drugs that show promise in vivo will be examined in vivo using [75Se] labelled cercariae. Drugs that we have screened and results are:

Drug	Inhibition	pH	mM	Inhibition
Caffeic acid	lipoxigenase	7.0	1	No effect
		7.0	10	No effect
		6.0	1	No effect
Ketoconazole	5-lipoxigenase	7.0	0.1	94%
		7.0	0.001	91%
		7.0	0.0001	31%

Caffeic acid has been reported in the literature as a slightly more potent lipoxigenase inhibitor than esculetin. However, our in vitro screening has shown it to be ineffective in inhibiting or suppressing cercarial stimulation by linoleic acid. On the other hand, Ketoconazole was very effective in vitro and has recently been reported to be a specific inhibitor of 5-lipoxigenase. Since we have shown that 5-lipoxigenase products, particularly LTB<sub>4</sub>, are involved in cercarial penetration, the effect of ketoconazole in vitro may be particularly significant. In addition, ketoconazole is already in use as an antifungal agent with known pharmacokinetics. During the coming year we intend to continue to screen compounds that have been reported as either potent lipoxigenase inhibitors or having some anti-schistosomule activity. Those compounds to be screened are: ETYA, ivermectin, cis-retinoic acid, trans-retinoic acid, NDGA, tetradecanoic acid, imidazole, sulfasalazine, propylgalate, amoscanate, cyclosporine, and praziquantel. Compounds that show effectiveness in vitro will be tested in vivo.

#### 9. Preliminary Experiments on the Use of an Artificial Skin Membrane to Investigate Cercarial Penetration and Transformation Mechanisms in vitro.

We have obtained permission to utilize a patented process for the manufacture of an artificial skin membrane. This membrane is a chitin:keratin, collagen:keratin or a chitin:collagen: keratin polymer. This polymer can be varied in size and thickness. In addition, various substances such as linoleate and/or drugs can be incorporated into the polymer. Preliminary experiments showed that cercariae were not able to penetrate through membranes made using the patented formula. We plan to continue experimentation with

test membranes by varying thickness, pH and composition. In addition, we are experimenting with a gelatin membrane described by Clegg for use with bird schistosome species. Our goal is to develop an artificial transformation method that can result in true schistosomes and have a more "natural" method to test drugs in vitro.

TABLE I  
 Cercarial/Schistosomular Characteristics After 4Hrs Incubation Using Various Transformation Methods

Transformation Method (4hrs incubation)	Dye Uptake		EM		Granule Migration	Eicosanoids		
	Buffer	Water Tolerance	Glycocalyx Membrane	Surface Membrane		Nuclear Condition	(ng/10,000 cercariae) PGE 5-HETE	LTB4
3M Linoleate	Elac	90.00%	A	D	H	12.38	16.48	8.38
	Saline	88.84%	A	D	H	14.29	44.57	17.71
Heat	Elac	8.36%	I	D	H	0.12	3.96	1.54
	Saline	0.86%	P	S	H	0.22	7.21	0.45
Mechanical	Elac	15.00%	A	D	H	0.14	2.89	1.32
	Saline	4.13%	P/A	S/D	H	0.37	6.69	1.23
3M Linoleate + Mechanical	Elac	13.41%	A	D	H	1.67	8.54	6.21
	Saline	71.14%	A	D	H	6.77	16.03	8.20

Uptake of Trypan Blue dye indicates cercariae have lost their water tolerance (died)  
 A=Absent or reduced I=Intermediate P=Present P/A=Both types seen in different samples  
 D=Double membrane (heptalaminar) S=Single membrane (trilaminar)  
 H=Heterochromatic E=Euchromatic  
 (+) = degree of cyton granule involvement (-) = no cyton granules

## GLOSSARY

- Cercaria** - The infective stage of Schistosoma mansoni. This stage is released from the snail intermediate host and is the stage that infects the human host via skin penetration processes.
- Eicosanoid**- A generic term referring to both cyclo-oxygenase and lipoxygenase metabolites of arachidonic acid metabolism.
- ELAC** - Earles Salts with Lactalbumin hydrosylate.
- EM** - Electron microscopy
- HETE** - Hydroeicosatetraenoic acid.
- HPLC** - High Performance Liquid Chromatography.
- IP** - Intraperitoneal
- LT** - Leukotriene.
- LTB<sub>4</sub>** - Leukotriene B<sub>4</sub>.
- PG** - Prostaglandin.
- PGE** - Prostaglandin E.
- RIA** - Radioimmunoassay.
- RP** - Reverse phase
- Schistosoma mansoni** - (Trematoda) A human parasite infecting the mesenteric veins of its host. This parasite has a complex two host life cycle. Asexual reproduction occurs in an infected snail while the sexual (adult) stages occur in the human host. The infective stage for the definitive host (man) is the cercarial stage.
- Schistosomule** - A stage in the life cycle of Schistosoma mansoni. This stage occurs after the cercaria penetrates the host skin and undergoes biochemical and morphological processes called transformation.

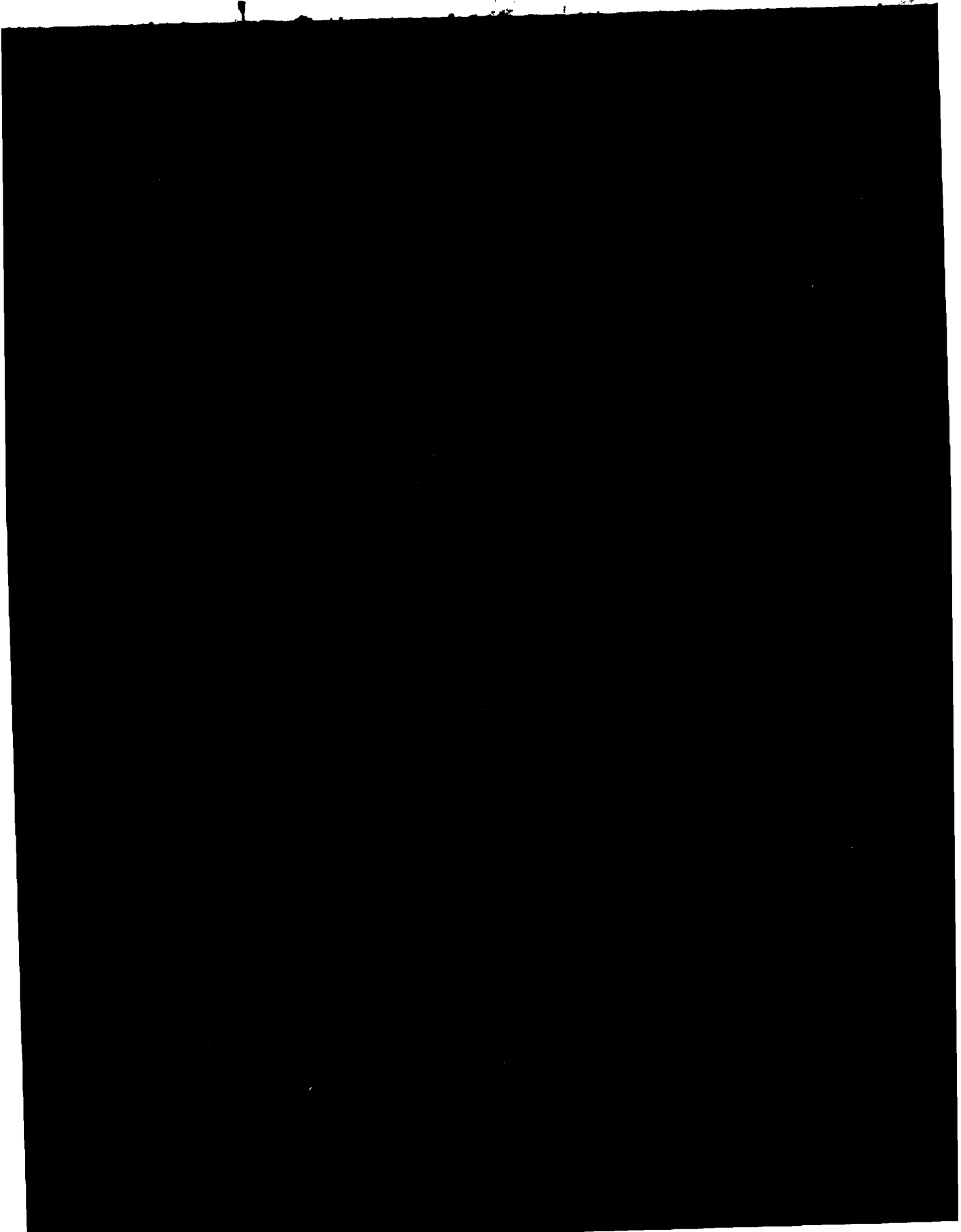
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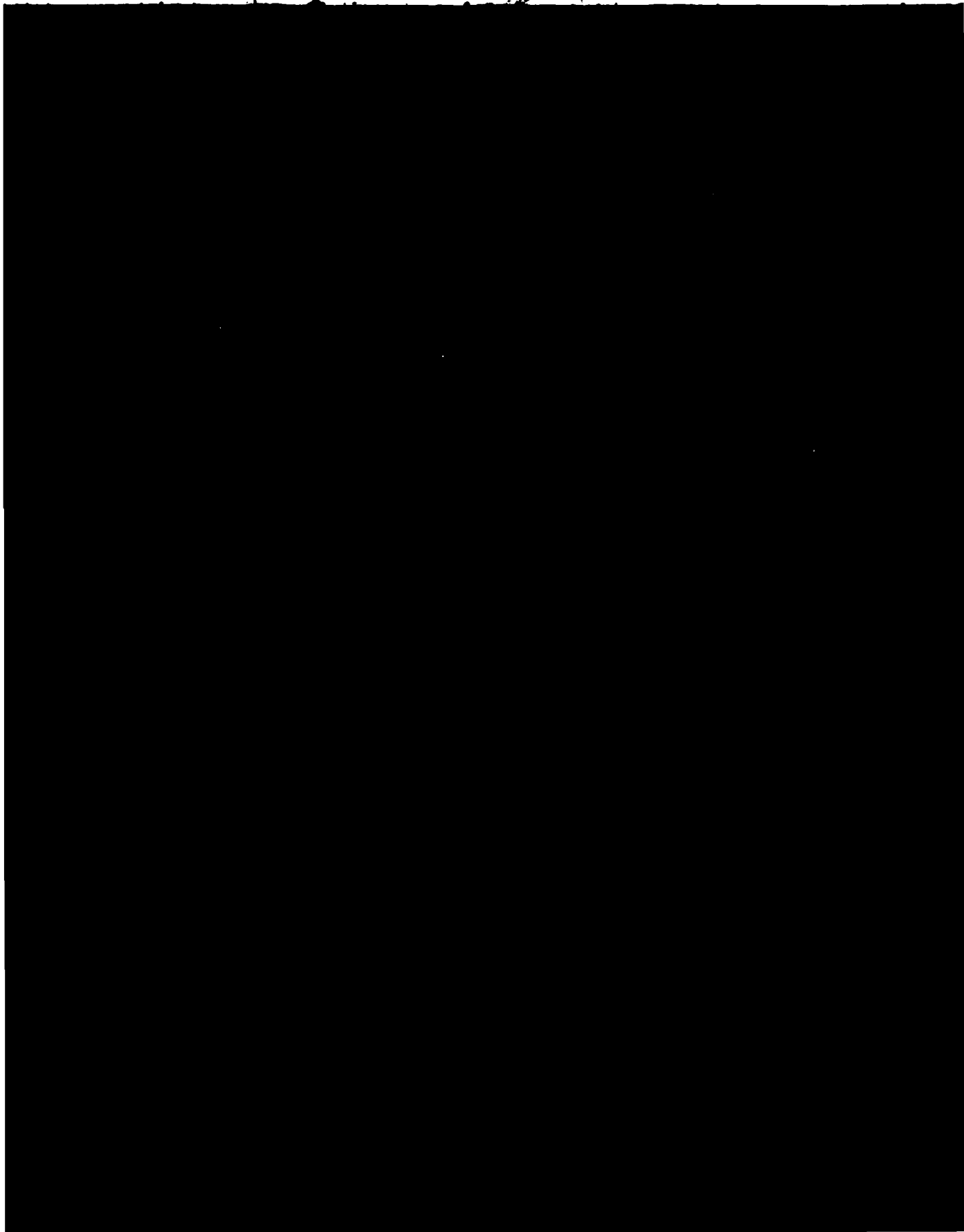
2. Role of pH in Cercarial Eicosanoid Production.

We have noted that the cercarial transformation process is pH sensitive. Cercarial penetration through an agar:gelatin matrix containing 3mM linoleate



tion and Transformation in vivo.

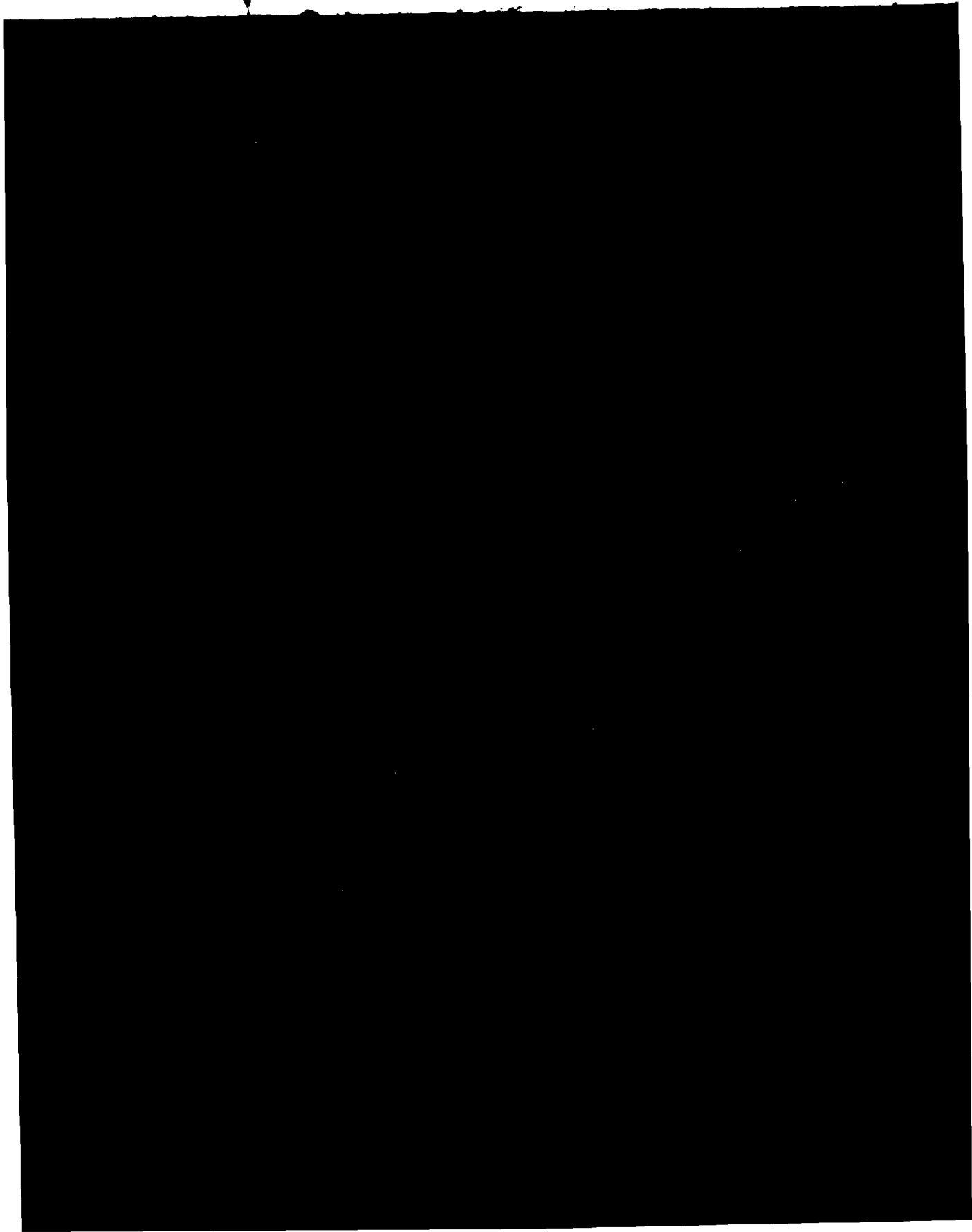
Since obtaining our [75Se] license we have run numerous studies evaluating the technique of using [75Se] labelled cercariae for the measurement of cercarial penetration and migration. In our hands, using the ICR mouse strain between 78.5 and 87.9% of labelled cercariae penetrate mouse tail skin. Of these 77.6% are found in the lungs at 7 days, and 18.5% remained in tail



that a one time dose of esculetin can be an effective chemoprophylactic agent.

b) Tracking of [75Se] Labeled Cercariae in Untreated Mice. See #4 above.

c) The role of esculetin as a prophylactic inhibitor of cercarial penetration. Esculetin (100mg/kg) was injected IP into 18 mice. Five mice were



We are just finishing a series of experiments investigating various cercarial transformation methods with respect to loss of water tolerance, tail loss, eicosanoid production, ultrastructure and RNA, DNA and protein synthesis. We have completed those studies involving loss of water tolerance, eicosanoid production (via RIA) and ultrastructure (via EM), but not those involving RNA, DNA and protein synthesis. Four transformation methods have been utilized in this study: chemical stimulation by 3mM Linoleate, mechanical shearing of tails, chemical/mechanical combination and incubation in buffer.

