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THE EFFECTS OF DIFFERENT GASES ON EXCYSTATION OF COCCIDIAN OOCYSTS

A Thesis

Presented to the

Department of Zoology

Brigham Young University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

James B. Jensen

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This thesis by James B. Jensen is accepted in its present form by the Department of Zoology of Brigham Young University as satisfying the thesis requirement for the degree of Master of Science.

Typed by Joyce M. Townsend

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INTRODUCTION

Generally, the term coccidiosis refers to a disease caused by intracellular parasites of the genera *Eimeria* and *Isospora*. Excystation, as applied to the coccidia, is a diphasic process by which sporozoites are released from the sporocysts and oocysts. Phase I consists of alterations in oocyst wall, either biochemically or mechanically, and Phase II the release of the sporozoites under the influence of trypsin and bile. Previous investigations have shown that CO_2 and a suitable reducing agent can change the oocyst wall permeability allowing the entrance of trypsin and bile (Jackson, 1962). The mechanism of CO_2 action, however, is not well understood.

The purpose of this project was to indirectly investigate the relationship between CO_2 and in vitro excystation of coccidia by investigating the effects of other gases on excystation. It was proposed that if another gas, or gases, could be found which would mimic the action of CO_2 in excystation, an examination of properties common between such gases and CO_2 might increase our understanding of the role of CO_2 in this process.

Coccidia from three different host animals were chosen for this study: *Eimeria stiedae* (rabbit), *Eimeria bovis* (cattle) and

Eimeria tenella (chicken). The following gases: N_2O, NO_2, NO, SO_2, NH_3, CH_4 and H_2S were tested as to their effects on excystation of the above named parasites.

LITERATURE REVIEW

Carbon dioxide has been found to be an important factor in the activation of many species of larval parasites. Rogers and Sommerville (1960) found that CO_2 was essential to the exsheathment of nematode larvae, and Graff and Kitzman (1965) reported that activation of acanthocephalan cystacanths was markedly stimulated by the presence of CO_2. In vitro studies of the excystment of *Fasciola hepatica* have shown that activation of the metacercaria occurred under the influence of CO_2 (Dixon, 1966), and Gallie and Sewell (1970) reported that the hatching of cestode eggs (*Taenia saginata*) was highly successful only after initial exposure to CO_2. Likewise, the activation of coccidian sporozoites is greatly influenced by CO_2 (Bunch and Nyberg, 1970).

The sporulated coccidian oocyst (Eimeriidae) contains eight potentially infective sporozoites in a dormant state. Early investigations into the process of excystation were conducted by Smetana (1933) who reported the influence of pancreatic enzymes on *in vitro* excystation of the rabbit coccidium, *Eimeria stiedae*. He designated trypsin as the specific enzyme involved since others tested had no effect. Goodrich (1944) was unable to repeat the work of Smetana

unless the oocysts of *Eimeria stiedae* were mechanically broken. She pointed out that the oocysts in Smetana's experiment had been collected from materials which had been ground in a mortar, and which probably contained many broken oocysts. Investigations by Koyama (1956) showed that oocysts of *Eimeria tenella* were not susceptible to the action of trypsin unless released first by mechanical alteration of the oocyst wall. Doran and Farr (1962) reported that mechanical breakage of the oocyst was essential to successful excystation of the coccidia of gallinaceous birds. Lotze and Leek (1960) using three coccidia species from sheep, reported that bile in combination with trypsin greatly enhanced the process of excystation.

Following the procedures that Rogers and Sommerville (1960) used in nematode exsheathment, Jackson (1962) successfully obtained excystation with *Eimeria arloingi* by using CO_2 and a reducing agent prior to incubation with trypsin and bile. He suggested that the process of excystation required two different stimuli; the first occurring in the rumen and the second in the small intestine. Nyberg and Hammond (1969) obtained *in vitro* excystation of *Eimeria bovis* oocysts by first treating them with CO_2 and the reducing agent, cysteine hydrochloride, followed by trypsin, steapsin and bile. Nyberg et al. (1968), working with *Eimeria tenella*, showed that the mechanical breakage of the oocysts was not necessary if they were incubated under an atmosphere of CO_2 in the presence of a reducing

agent. Bunch and Nyberg (1970), using oocysts from five different hosts: *Eimeria stiedae* (rabbit), *Eimeria acervulina* (chicken), *Eimeria scabra* (swine), *Eimeria intricata* (sheep) and *Isospora canis* (dog), were able to show that CO_2 and cysteine hydrochloride provided the necessary initial stimulus for excystation in a wide variety of coccidian species.

The importance of CO_2 and reducing agents is further demonstrated by the fact that in every coccidian species in which they were used excystation levels were markedly increased. Likewise, in every species tested no other initial stimulus was necessary if the incubation temperature is maintained between 34-41° C. The fact that larval parasites from four different phyla (Acanthocephala, Protozoa, Platyhelminthes, and Nemathelminthes) are activated under the influence of CO_2 suggests that it is an important biochemical reaction.

MATERIALS AND METHODS

Collection and Preparation of Oocysts

Oocysts of *Eimeria stiedae* were collected from two rabbits which had been given a sublethal dose of 25,000 oocysts each. Likewise, the oocysts of *Eimeria tenella* were collected from several chickens given 30,000 oocysts each. Oocysts of both species were separated from bulk fecal materials by washing the collected feces through a series of Tyler sieves from 60 mesh through 270 mesh. The screened materials were concentrated by sedimentation and decantation. The oocysts were further cleaned of residual debris by centrifugation in a continuous 50:50 sucrose-water gradient which was prepared by an ISCO Model 570 gradient former. The cleaned oocysts were suspended in 2.5% potassium dichromate solution in a 500 ml beaker and stirred continuously by a Cole-Palmer rotary mixer for several days to facilitate sporulation. After sporulation was completed the oocysts were stored in the 2.5% potassium dichromate solution at 4° C until used. Clean oocysts of *Eimeria bovis* were supplied by Dr. Datus M. Hammond, Utah State University, Logan, Utah.

Preparation of Reagents

The excysting solutions were prepared by adding 1 gram of trypsin 1-300 Nutritional Biochemical , 10 g of bovine bile Bacto-oxgall, Difco and 0.85 g of NaCl to 100 ml of distilled water. The resulting concentrations were 1% trypsin, 10% bile and 0.85% saline. These solutions were made up in a 0.2M phosphate buffer at pH 7.0 and then adjusted to pH 7.6 by addition of 1.0N NaOH. The entire mixture was divided into 3.5 ml portions and stored at -20° C until used. Immediately before use, this solution was thawed and added in proportions of 1:1 with a suspension of oocysts. The cysteine hydrochloride solution (.04 M) was prepared fresh for each experiment. The sodium hypochlorite was procured under the trade name of Chlorox^{R}. The gases: CO_2, N_2O, NO, N_2, CH_4, SO_2, NH_3, and H_2S were obtained in cylinders from Matheson Company, Inc., Haywood, Calif.

Procedure for Excystation

Normally excystation is accomplished by first exposing the oocysts to CO_2 and the reducing agent followed by incubation in trypsin and bile (Nyberg and Hammond, 1964). In this project the following gases were used in place of CO_2: N_2O, NO_2, NO, CH_4, SO_2, NH_3 and H_2S. Carbon dioxide and nitrogen were used as positive and negative controls, respectively.

Oocyst suspensions were rinsed free of potassium dichromate by repeated washing and centrifugation with distilled water. The oocysts were then resuspended in distilled water and mixed 1:1 with .04M cysteine hydrochloride in 125 ml erlenmeyer flasks fitted with serum stoppers. The flasks were flushed with the respective gases for two minutes. Since nitric oxide rapidly oxidizes to nitrogen dioxide in the presence of oxygen it was necessary to pre-treat these flasks with nitrogen to remove all the atmosphoric oxygen. The nitrogen dioxide was generated by simply allowing the nitric oxide to oxidize to nitrogen dioxide. After the flasks were filled with the respective gases they were incubated in a Lab-Line waterbath at the following times and temperatures: *Eimeria stiedae* for 2 hours at 36° C, *Eimeria bovis*, 8 hours at 39° C, and *Eimeria tenella*, 18 hours at 39° C. Although some investigators found other time and temperature combinations to be optimal for excystation (Hibbert and Hammond, 1968) these differences were not considered to be critical.

Determination of Oocyst Wall
Permeability Changes

Activation. After the appropriate incubation with the test gas and reducing agent the oocysts were rinsed with distilled water, recovered by centrifugation, resuspended in distilled water and mixed 1:1 with trypsin and bile. The pH was adjusted to 7.6 with NaOH and the suspension was reincubated at the same temperatures

used during the test gas treatment. Wet mount slides were prepared and examined at 1/2 hour intervals for up to six hours. The percentage of activation was determined by counting the number of completely and partially excysted oocysts for every 100 completely sporulated oocysts. It was found that after six hours it was difficult to distinguish oocysts which were empty as the result of complete excystation or empty as the result of the digestive action of trypsin and bile on dead oocysts.

Staining with methylene blue. Since the oocyst wall is impermeable to histo-chemical dyes unless altered either biochemically or mechanically, the penetration of such dyes was considered a positive indication of changes in oocyst wall permeability. After proper incubation with gas and reducing agent, the oocysts were washed and suspended in methylene blue for up to 1/2 hour. The staining of the oocyst inner wall and internal structures was considered a positive test.

Treatment with sodium hypochlorite. When oocysts are suspended in 5.5% sodium hypochlorite, the outer wall is removed but there is no apparent effect on the inner wall. After treatment with CO_2 and a reducing agent the integrity of the inner wall is disturbed after which it can also be removed by suspension in sodium hypochlorite (Jackson, 1962). After the gas-reducing agent treatment the oocysts were

washed and suspended in sodium hypochlorite for up to 1/2 hour and subsequently examined for the removal of the inner wall. The removal of the inner wall was considered another positive indication of changes in oocyst wall permeability.

Treatment with 8M Urea

One final experiment was conducted using an 8M urea solution in place of CO_2. This solution is commonly used in protein denaturation experiments, and since the changes in oocyst wall permeability appear to be caused by changes in the protein structure it was decided that the effects of 8M urea would be informative. Eight molar urea was used alone and in various combinations with CO_2 and cysteine hydrochloride. The oocysts were then tested for their abilities to excyst, and their susceptibility to sodium hypochlorite and methylene blue staining.

Selection of Gases

Various test gases were chosen to hopefully elucidate the mechanism of CO_2 action. Since aqueous solutions of CO_2 are acidic in nature, gases which were also acidic in aqueous solutions were chosen. These included, SO_2, NO_2 and H_2S. Dinitrogen oxide (N_2O) was selected because it has physical properties identical to CO_2 such as: dipole moment (non polar), molecular weight and

density. Nitric Oxide (NO) is smaller than CO_2 but is more reactive, having an extra electron. Methane (CH_4) was chosen because it is a completely reduced form of carbon and is found in high concentration in the gut of many animals. Ammonia (NH_3) was selected because it is found in dung piles and chicken coops, etc., where coccidia would also be found.

Since early experiments were unsuccessful in producing excystation, it was decided to test the toxicity of the test gases by first exposing the oocysts to the test gas and then subjecting them to CO_2 and cysteine hydrochloride, followed by trypsin and bile.

Failure to excyst normally after the CO_2-cysteine hydrochloride treatment was considered an indication of toxicity or competitive inhibition by the test gas.

RESULTS

The results obtained for the 3 species of coccidia tested, *Eimeria stiedae*, *Eimeria bovis*, and *Eimeria tenella* are outlined in tables 1, 2 and 3 respectively. In general the gases can be grouped into three categories: those which had no apparent effects on the oocysts, those which were toxic, and those which caused changes in oocyst wall permeability. The results obtained with 8M urea are discussed, but not tabulated.

Dinitrogen oxide (N_2O)

Dinitrogen oxide was not effective in inducing oocyst wall permeability as shown by the fact that treatment with trypsin and bile did not result in activation. Methylene blue did not stain the inner wall of the oocysts or the sporocysts, and treatment with sodium hypochlorite did not affect the inner wall. All three species: *Eimeria stiedae*, *Eimeria bovis*, and *Eimeria tenella* were used with exactly the same results. Subsequent exposure to CO_2 and cysteine hydrochloride after incubation in N_2O showed normal activation.

Table 1. Effects of gases on <u>Eimeria stiedae</u> as determined by sporozoite activation, internal staining with methylene blue, and effect of sodium hypochlorite on the inner oocyst wall following treatment.

Gases and cysteine HCl	Activation	Chlorox	Methylene blue stain	Toxic effect
CO_2	100%	+	+	-
N_2O	0%	-	-	-
NO_2	10%	-	-	-
NO	30-40%	+	+	-
CH_4	0%	-	-	-
SO_2	0%	-	-	+
NH_3	0%	-	-	+
H_2S	50%	+	+	±*

*Under some conditions, outlined in the text, H_2S may be mildly toxic.

Table 2. Effects of gases on <u>Eimeria bovis</u> as determined by sporozoite activation, internal staining with methylene blue, and effect of sodium hypochlorite on the inner oocyst wall following treatment.

Gases and cysteine HCl	Activation	Chlorox	Methylene blue stain	Toxic effect
CO_2	60%	+	+	-
N_2O	0%	-	-	-
NO_2	0%	-	-	-
NO	10%	+	+	-
CH_4	0%	-	-	-
SO_2	0%	-	-	+
NH_3	0%	-	-	+
H_2S	20%	+	+	±*

*Under some conditions, as outlined in the text, H_2S may be mildly toxic.

Table 3. Effects of gases on *Eimeria tenella* as determined by sporozoite activation, internal staining with methylene blue, and effect of sodium hypochlorite on the inner oocyst wall following treatment.

Gases and cysteine HCl	Activation	Chlorox	Methylene blue stain	Toxic effect
CO_2	50%	+	+	-
N_2O	0%	-	-	-
NO_2	0	-	-	-
NO	10%	+	+	-
CH_4	0%	-	-	-
SO_2	0%	-	-	+
NH_3	0%	-	-	+
H_2S	20%	+	+	$\pm$*

*Under some conditions, as outlined in the text, H_2S may be mildly toxic.

Nitrogen dioxide (NO_2)

This gas did show a low percentage of excystation (10%) with Eimeria stiedae but no excystation was noted with Eimeria bovis or Eimeria tenella. The staining with methylene blue and treatment with sodium hypochlorite was negative in all three species. The gas was not toxic to the oocysts as excystation occurred normally with CO_2 and cysteine hydrochloride following the initial NO_2 exposure.

Nitric oxide (NO)

This gas caused changes in oocyst wall permeability as is evident in that some activation occurred with all three species. Eimeria stiedae showed 30-40% activation, whereas Eimeria bovis and Eimeria tenella were activated at a level of less than 10% each. All three species showed a positive staining test. Eimeria bovis was the most susceptible to staining with methylene blue. Treatment with sodium hypochlorite following incubation of the oocysts of all three species in NO and cysteine hydrochloride indicated a change in permeability as the outer and inner oocyst walls were both removed leaving a thin membrane around the four sporocysts and many free sporocysts in the solution. Nitric oxide was not toxic.

Methane (CH_4)

This gas did not change the oocyst wall permeability. Acti-

vation, staining, and sodium hypochlorite tests were negative with all three species. Methane (CH_4) is not toxic to the oocysts.

Sulfur dioxide (SO_2)

Sulfur dioxide was apparently toxic to the oocysts of all three species. Activation did not occur after initial treatment with SO_2, and treatment with CO_2 and cysteine hydrochloride following exposure to SO_2 did not bring about activation. The oocysts' internal components would not stain with methylene blue after treatment with SO_2, but after first exposure to SO_2 followed by CO_2 and the reducing agent the stain did penetrate the oocyst. From this observation it is evident that SO_2 is toxic to the sporozoites but does not compete with CO_2 for a specific binding site, if one exists.

Tests with sodium hypochlorite were negative with all three species following treatment with SO_2.

Ammonia (NH_3)

Like SO_2 this gas was toxic to the oocyst and its contents. Activation, staining and sodium hypochlorite tests were all negative. Subsequent activation of sporozoites with CO_2 and cysteine hydrochloride was not successful following exposure to NH_3. Ammonia, while it did kill the sporozoites, did not prevent oocyst wall permeability changes with exposure to CO_2 and cysteine as was

evident by the staining of sporocysts and oocyst inner walls following NH_3 and CO_2 incubation with the reducing agent. Sodium hypochlorite also removes both walls of the oocyst following exposure to both gases but not to NH_3 and cysteine hydrochloride alone.

Hydrogen sulfide (H_2S)

Hydrogen sulfide was successful in changing oocyst wall permeability. Activation occurred with all three species following exposure to H_2S and cysteine hydrochloride and trypsin and bile. *Eimeria stiedae* showed sporozoite activity in up to 50% of the oocysts. *Eimeria bovis* and *Eimeria tenella* also were affected by H_2S, as activation occurred in up to 20% of the oocysts of these two species. It should be mentioned, however, that while many sporozoites successfully escaped the sporocysts only a few were seen outside the oocyst. It was also noted that some sporozoites were shorter in length and thicker than those seen in CO_2 stimulated excystation. Many were normal looking, however.

When the oocysts of *Eimeria stiedae* were incubated with H_2S alone, without the reducing agent, sporozoite activation was noted in up to 25% of the oocysts. Prolonged incubation or incubation at temperatures of 41° C or higher with H_2S and cysteine hydrochloride quickly lowered activation levels. It appears that H_2S may be toxic under these conditions.

Following treatment with H_2S and cysteine hydrochloride the methylene blue would diffuse into the oocysts, and 5.5% sodium hypochlrite would remove the outer and inner walls of the oocyst of all three species.

8M Urea

Experiments revealed that 8M urea was weakly toxic to the oocysts of all three species. When used alone or with cysteine hydrochloride, no excystation was achieved. When used with CO_2 and cysteine hydrochloride, excystation levels were only 20% (as opposed to 85% in the controls). When urea was incubated with the oocysts followed by CO_2 and cysteine hydrochloride, no activation was noted. The staining and sodium hypochlorite tests were also negative.

DISCUSSION

As was stated earlier, some gases did not appear to have any effect on the oocysts at all, others were toxic and two gases were evidently able to cause changes in oocyst wall permeability.

Both dinitrogen oxide (N_2O) and methane (CH_4) gave negative results without being toxic. It is not known whether these gases penetrated the oocyst or whether they were simply ineffective in changing oocyst wall permeability. Both gases are characteristically inactive, however, and it is not difficult to concede that they simply have no apparent effect on coccidian oocysts.

The small amount of activation noted with nitrogen dioxide (NO_2) on *Eimeria stiedae* might be explained by the method of preparation of the gas in this experiment. When nitric oxide (NO) is exposed to atmospheric oxygen, it rapidly oxidizes to nitrogen dioxide (NO_2). Nitric oxide was allowed to oxidize to obtain the nitrogen dioxide used. Since nitric oxide does show some effect on the oocyst wall permeability, the activity seen with nitrogen dioxide was probably caused by residual nitric oxide. Except for this instance NO_2 does not appear to affect the oocysts in any way.

The toxic effects of SO_2 were unexpected because the inorganic

acids of HNO_3, HCl, and H_2SO_4 will not penetrate the oocyst wall in any concentration incapable of breaking them (Kheysin, 1972). It would be expected that the SO_2 solution in water, sulfurous acid (H_2SO_3), would likewise have no effect. It may be that the SO_2 diffuses through the oocyst wall forming H_2SO_3 with the intraoocyst fluid, which is then toxic. It may be that the increase in hydrogen ion concentration within the oocyst is responsible for this toxicity and that such an increase does not stimulate activation of an excysting enzyme or enzymes. One should not rule out the possibility of direct action of SO_2 (not H_2SO_3) in producing the toxic effect.

The toxic effects of ammonia (NH_3) observed in this project are supported by the works of Petrov and Nikonov (1964) who report that solutions of ammonia penetrate the oocysts of *Eimeria zurnii* and after ten minutes rendered them unable to sporulate. Horton-Smith et al. (1940) also reported that a 1% solution of ammonia killed 100% of the oocysts of *Eimeria tenella* in 24 hours, and a 5% solution was lethal in 2 hours and a 10% solution in 45 minutes.

Before starting this project it was proposed that if a gas, or gases, could be found which would mimic the action of CO_2, i.e., to cause changes in oocyst wall permeability, an examination of properties held in common with CO_2 might reveal the key to the mechanism of CO_2 action. As seen in the results of this experiment, two other gases, NO and H_2S, do cause changes in oocyst wall

permeability. Except for the fact that all three are gases there is little relationship between CO_2, NO, and H_2S. Perhaps an explanation can be found by reviewing the reported attitudes towards CO_2 as they relate to excystation of the coccidia. Jackson (1962) stated that CO_2 did indeed cause changes in oocyst wall permeability and that this action was greatly enhanced by the introduction of a reducing agent. Nyberg et al. (1968) suggests that the action of CO_2 is a general and important phenomenon in the activation of larval parasites. Bunch and Nyberg (1970) state that two factors are known to satisfy the initial stimulus in excystation, mechanical stress and CO_2. Thus it appears that the major emphasis was on CO_2 rather than the reducing agent in this reaction. The suggestion was that the gas is aided by the reducing agent, rather than the gas aids the reducing agent. Close examination of Jackson's paper shows that the oocysts of *Eimeria arloingi* were incubated in rumen fluid under an atmosphere of CO_2. Later he states that the addition of a reducing agent increased the level of excystation. It is very possible that the rumen fluid contained a natural reducing agent which was only aided by the addition of another reducing agent. Rogers and Sommerville (1960) state that deactivated rumen fluid can be revitalized to stimulate nematode exsheathment by the addition of a reducing agent. Nyberg et al. (1968) and Bunch and Nyberg (1970) both used a reducing agent in their *in vitro* studies. Hence it appears that the gas, CO_2, is aiding the reducing agent, rather than vice versa.

Although NO and H_2S do not appear to be related to CO_2, they are related to the reducing agents. Hydrogen sulfide can donate its hydrogen in an oxidation-reduction reaction and nitric oxide can donate an electron in similar reactions.

The importance of the reducing agent in changing oocyst wall permeability is further explained by Dr. Sheril Burton (personal communication, 1972). He explained that an assay for sulfhydryl groups on the oocyst wall showed only a very few to be present. After treatment with CO_2 and a reducing agent, however, the number of sulfhydryl groups was greatly increased. Apparently, there are many disulfide bridges in the protein structure of the oocyst wall which aid in maintaining its semi-permeable condition. These bridges are reduced to sulfhydryl groups by the action of the reducing agent thereby causing a change in the permeability of the wall. Hence the role of CO_2 in excystation may be that of an allosteric effector. By combining with a protein molecule and causing slight changes in its configuration the CO_2 may render the disulfide bridges more susceptible to the action of the reducing agent. The small size and ability to diffuse into the wall may explain why NO and H_2S are able to change the wall structure by their reducing properties. This, too, may explain why H_2S will work without cysteine hydrochloride being present.

Although 8M urea is used in denaturing proteins, its action is

not on the disulfide bridges, but rather on the hydrogen bonding which hold protein molecules in their specific configurations. The fact that 8M urea did not cause permeability changes in the oocyst wall leads one to believe that it is indeed disulfide bridges, and not hydrogen bonding, which is responsible for the semi-permeable condition of the oocyst wall. Eight molar urea did, however, prevent the CO_2 and reducing agent from changing the wall. Perhaps the 8M urea denatured the receptor site on the protein molecule where CO_2 exerts its allosteric effect.

Another possible explanation to the mechanism of CO_2 action may be seen by making reference to the work of Monné and Hönig (1954). In their study of the properties of oocyst walls they stated that the apparent inability of sodium hypochlorite to remove, or influence the inner wall, was perhaps due to the protection of the protein by its lipid contents. In light of this statement, and the observation that the inner wall is indeed affected by sodium hypochlorite after CO_2 and reducing agent treatment, it is possible that CO_2 causes changes in the interrelationship between the protein and the lipid micelles of the oocyst wall. Such changes may remove the protection of the lipid micelles and uncover the disulfide bridges to the action of the reducing agent. Likewise, the gases NO and H_2S may diffuse into the lipid and cause reduction of the disulfide bridges regardless of such apparent protection.

SUMMARY

An indirect approach to the study of the mechanism of CO_2 action in excystation of coccidian oocysts was undertaken by substituting several gases in place of CO_2. The objective was to find gases other than CO_2 which would mimic the action of CO_2. Of the gases tested; NO, NO_2, N_2O, H_2S, CH_4, SO_2 and NH_3, only NO and H_2S showed reactions similar to CO_2 in causing sporozoite activation. Both of these gases have reducing properties which underscore the importance of reducing agents in the over all mechanism of excystation. It now appears that the role of CO_2 may be that of an allosteric effector which aids the reducing agent in breaking up the disulfide bonds which are responsible for the semi-permeable state of the oocyst wall.

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THE EFFECTS OF DIFFERENT GASES ON EXCYSTATION

OF COCCIDIAN OOCYSTS

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ABSTRACT

Excystation of coccidian oocysts is a diphasic process where CO_2 and suitable reducing agents cause changes in oocyst wall permeability (phase I) allowing the entrance of trypsin and bile which activate the sporozoites (phase II). This project was an indirect study of the mechanism of CO_2 action by the substitution of NO, NO_2, N_2O, H_2S, SO_2, CH_4, NH_3 and 8M urea in place of CO_2. Changes in oocyst wall permeability of *Eimeria stiedae*, *E. bovis* and *E. tenella* were determined by incubation with the reagents and cysteine HCl followed by treatment with trypsin and bile to initiate activation of sporozoites, staining oocyst inner structures with methylene blue, and removal of outer and inner oocyst walls with sodium hypochlorite.

The gases CH_4, NO_2, N_2O were negative for all 3 tests as were SO_2, NH_3 and 8M urea which in addition were toxic to the oocysts. Both H_2S and NO were capable of mimicing the action of CO_2 and are related chemically to the reducing agent, and hence tend to underscore its importance in excystation. It now appears that the role of CO_2 is that of an allosteric effector enhancing the action of the reducing agent.

COMMITTEE APPROVAL: ______________________________