naeus robustus, with work on the difficult problem of the development and methods of use of effective and economical gear in deep water.

LITERATURE CITED

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Nueva Información Sobre los Camarones Rojos en el Golfo de Méjico

STEWART SPRINGER

U. S. Fish and Wildlife Service, Pascagoula, Miss.

Abstracto

Se discute la importancia de las poblaciones de camarones, tanto los que están en uso ahora, como los que se encuentran en cantidades potenciales. Se resumen los resultados de dragas hechos en aguas profundas por el bajel Oregon, incluyendo el trabajo hecho cerca de Dry Tortugas, Florida, en profundidades de 200 a 300 brazas, donde las pescas de camarones de aguas profundas, *Hymenopenaeus robustus,* indicaron una distribución irregular y la posibilidad de que ocurren en grandes concentraciones.

A Practical Method of Dispersing Aureomycin* Chlortetracycline In Ice

S. D. UPHAM, F. E. STIRN, J. F. WEIDENHEIMER, F. M. CALLAHAN AND L. RITTER

American Cyanamid Company, Research Division, Pearl River, N. Y.

The obvious commercial and economic advantages to be gained by the use of any agent which would extend the storage period of organic materials liable to spoilage, has directed the interest of many workers toward this field. After several years of extensive work Dr. H. L. A. Tarr[1] and colleagues have concluded "that chlortetracycline was more effective in preserving such foods (meats and fish) than any other of fourteen antibiotics studied". Others who have worked in this field are E. A. Fieger[2], C. P. Idyll[3], and F. E. Deatherage[4]. Early investigators demonstrated that when

* *The trade mark of the American Cyanamid Company for the Antibiotic Chlortetracycline is Aureomycin.*

solutions of antibiotics were frozen, severe migration towards the center of the ice cake occurred. This problem was overcome for experimental purposes by the use of flake ice[1] or by freezing thin layers of the solution in shallow trays and crushing with an "Ice Queen" crusher[2]. However, since block ice represents 95% of the commercial form it was of prime importance that a method be found which could be applied to the existing commercial ice manufacturing systems with a minimum of change. Consequently, investigations along this line were initiated in this laboratory which culminated in the development of a feasible commercial method.

The first approach was the use of various thickening agents to increase the viscosity of the solutions to be frozen, in the hope that this purely physical phenomenon would retard movement of the antibiotic towards the center of the ice cake. The importance of any retarding effect is shown when consideration is given to the extent of concentration of the antibiotic which develops when the antibiotic migrates two inches towards the center of a standard 300 lb. ice cake. In such an event, more than 50% of the ice will contain no antibiotic at all. If such a cake were crushed and used, erratic results could be expected. However, since in actual practice migration is almost 100% to the center, such ice would be completely worthless for the purpose intended.

A large number of thickening agents were systematically investigated. Since migration was prevented by some of these and not by others, it was readily apparent that a simple change in viscocity would not prevent this phenomenon of migration. For example, locust bean gum, polyvinyl pyrrolidone, acacia, agar agar and various types of starches were ineffective, while substances such as Irish moss (carrageen), carboxymethylcellulose, gelatine and algin controlled migration with varying degrees of efficiency. Closer examination of the different substances studied showed that those which were effective in controlling migration contained acidic groups which conferred on them salt forming properties with metals. When these successful systems were studied with distilled water instead of tap water, the migration was as severe as that experienced when antibiotic solutions were frozen without any added colloid. This led to the assumption that one or more metallic ions in the water were involved in the prevention of migration. This was proved to be true by employing systems using distilled water and adding various metal salts. It was then found that some of the metallic ions as shown in Table 1 were effective and that at least one or more of these ions were a necessary part of this system.

Previous work in this laboratory with polymeric materials, tetracyclines (particularly chlortetracycline) and metallic ions indicated that reactions involving complex formation, salt formation, or both, with the metal acting as a bridge between the polymeric substance and the tetracycline used, occurred quite readily under certain conditions. A similar type of reaction was assumed to occur here. This also led us to suspect that Aureomycin chlortetracycline would prove to be a most efficient antibiotic for the present purpose. Exhaustive experiments showed this to be the case.

The pH of the system plays an important role for it was demonstrated that below a pH of 7 efficiency decreased (Figure II). If the solution were too strongly alkaline, some potency losses might be expected.

The ice cake may then be pictured as having a gel structure of fairly

TABLE 1

EFFECT OF METALLIC IONS ON THE MIGRATION OF AUREOMYCIN CHLORTETRACYCLINE·HCL IN FROZEN SOLS

Unit Charge:

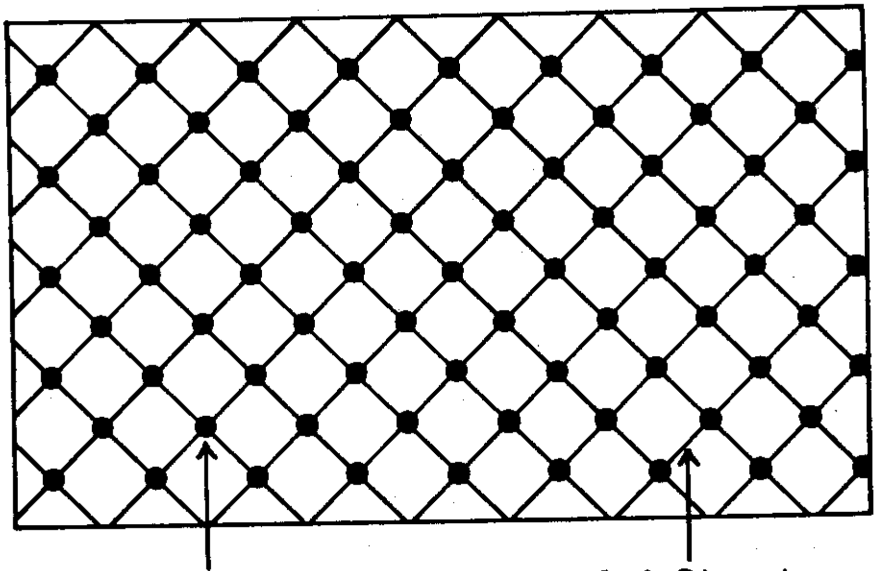
6 liters ....Distilled Water
1 gm. ....Carrageen (SeaKem No. 9)
180 mg. (30γ/ml.) ....Chlortetracycline·HCl

Metal		Added - gms.	% Retention in Outer Periphery
Aluminum	1.0	$Al(OH)C_2H_3O_2 \cdot xH_2O$	16
Barium	2.0	$Ba(OH)_2 \cdot 8H_2O$	11
Bismuth	1.0	$BiCl_3$	very low
Cadmium	1.0	$CdCl_2$	72
Calcium	0.25	$Ca(OH)_2$	71
Cerium	1.0	$Ce(NO_3)_3 \cdot 6H_2O$	very low
Cerium	1.0	$Ce(SO_4)_2$	32
Chromium	1.0	$CrCl_3$	very low
Cobalt	1.0	$CoCl_2 \cdot 6H_2O$	71
Copper	1.0	$CuCl$	very low
Copper	1.0	$CuSO_4 \cdot 5H_2O$	very low
Iron (Ferric Glycerophosphate	1.0	$Fe_2[C_3H_5(OH)_2PO_4]_3$	very low
Iron (Ferrous Gluconate)	1.0	$Fe[CH_2OH(CHOH)_4CO_2] \cdot 2H_2O$	very low
Iron	1.0	$Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$	complete
Iron	1.0	$Fe(NH_4)_2(SO_4)_2 \cdot 12H_2O$	98
Lithium (Lithium Citrate)	1.0	$C_6H_5Li_3O_7 \cdot 4H_2O$	very low
Magnesium	1.5	$MgCl_2 \cdot 6H_2O$	75
Manganese	1.0	$MnCl_2 \cdot 4H_2O$	complete
Mercury	1.0	$HgCl_2$	51
Molybdenum	1.0	$Na_2MoO_4 \cdot 2H_2O$	very low
Nickel	1.0	$NiSO_4 \cdot 6H_2O$	89
Potassium	1.0	KI	very low
Selenium	1.0	H_2SeO_3	very low
Silicon	1.0	$Na_2SiO_3 \cdot 5H_2O$	very low
Strontium	1.0	$SrCl_2 \cdot 6H_2O$	51
Tin	1.0	$SnCl_2 \cdot 2H_2O$	very low
Tin	1.0	$NaSnO_3 \cdot 3H_2O$	very low
Tungsten	1.0	$Na_2WO_4 \cdot 2H_2O$	very low
Vanadium	1.0	$NaVO_3 \cdot H_2O$	very low
Zinc	1.5	$ZnCl_2$	28
Zirconium	1.0	$ZrO_2 \cdot xH_2O$	very low
Zirconium	1.0	$ZrOCl_2 \cdot 8H_2O$	very low

The value of per cent retention in the outer periphery is the per cent of the theoretical value with complete inhibition of migration of the antibiotic towards the center. Where it was indicated to be very low, less than 10% of the original amount remained.

uniform consistency throughout with the antibiotic bound to this gel through the mediation of metal ion bridges as shown in the schematic drawing, Figure I.

Figure I



Experiment I

Chlortetracycline·HCl, Polyvinyl Alcohol and Calcium

The following materials were mutually dissolved in six liters of tap water containing about 0.25 gm. of calcium ion by analysis:

500 mg. ....Polyvinyl Alcohol (Elvanol 71-24)
180 mg. ....Chlortetracycline·HCl

The solution was placed in a freezing chamber and frozen at –10°C (14°F.) until completely solid. The frozen cake was removed and split in half from top to bottom along the diameter. An intense yellow core in the innermost section was disclosed, while the outer peripheries were devoid of all traces of chlortetracycline.

Chlortetracycline·HCl, CMC (Sodium Salt of Carboxymethylcellulose) in Tap Water

The following materials were dissolved in six liters of tap water by stirring with a mechanical stirrer until a homogeneous sol was obtained:

1.0 gm. ....Cellulose Gum (Hercules, Type 70-D, High)
180 mg. ....Chlortetracycline·HCl

The above sol was frozen at –10°C. until completely solid. On removal from the container wherein the sol was frozen, a cake of a uniform light yellow color was revealed. By fracturing the material into two equal sections along a diameter, this light yellow color was found to be distributed evenly throughout the cake. Over 80% of the theoretical amount of active material was found in the outermost section by spectrophotometric analysis.

Experiment II

A Sol of Chlortetracycline·HCl and CMC in Distilled Water

Six liters of a homogenous sol consisting of 180 mg. (30 γ/ml.) of chlortetracycline·HCl and 400 mg. of CMC were prepared in a routine manner. The pH of the material was determined to be 6.2. After this material was completely frozen in a container, the contents of the container were removed and examined. It was discovered that migration of the antibiotic to the center of the cake was extremely severe. By analysis, it was found that eleven times more antibiotic was concentrated in the center than was found in the outside portions of the ice cake.

A Sol of Chlortetracycline·HCl, CMC and Calcium in Distilled Water

The above experiment was repeated using 100 mg. of $CaCl_2$ in addition to the above formula. The total formula—

6 liters .... Distilled Water
180 mg. .... Chlortetracycline·HCl
400 mg. .... CMC (Carboxymethylcellulose)
100 mg. .... $CaCl_2$ (anhydrous)

The pH was again found to be 6.2. The analysis of the frozen cake now showed that the ratio of antibiotic in the innermost portion to the outside periphery was 1.3 to 1.0.

Experiment III

Chlortetracycline·HCl and Carrageen

It was expedient that a polymeric material which could be dissolved in cold water be found. Carrageen proved most suitable. 180 mg. of Chlortetracycline·HCl and 1.0 gm. of carrageen (SeaKem No. 9) were dissolved in six liters of tap water by stirring at room temperature. The sol was then completely frozen at –10°C. After removal of the cake from its container, the cake was inspected visually and it became easily discernible that a homogeneous dispersion had been obtained. Analysis of the innermost and outermost sections proved that a ratio of 1.0:1.0 existed.

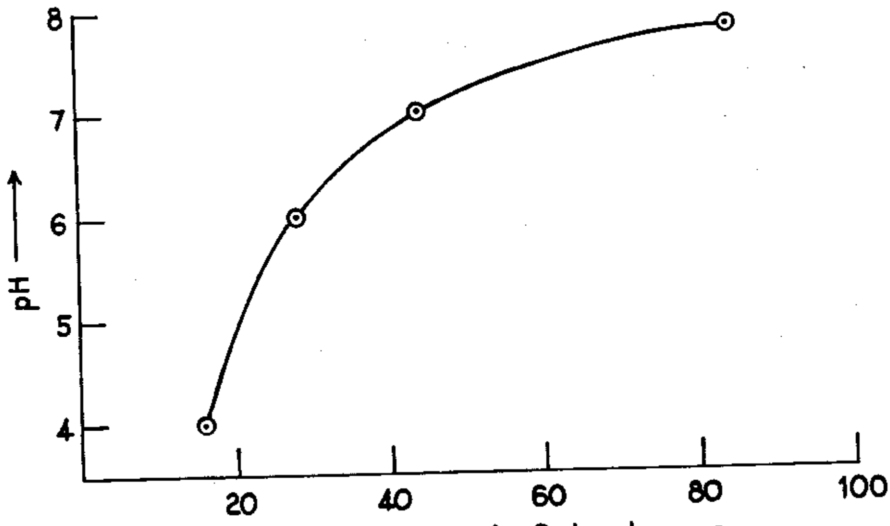
Experiment IV

pH Effect

A series of experiments was conducted to determine the effect of pH on migration of the chlortetracycline in a freezing sol. 500 mg. of carboxymethylcellulose (CMC) and 180 mg. of chlortetracycline·HCl were dissolved in six liters of tap water and the pH adjusted with hydrochloric acid. The use of organic acids instead of mineral acid did not alter the effect. The resultant sols were frozen and the outer layers analyzed. The results are given in Figure II.

Figure II

Effect of pH on Migration of CTC in Solutions



Commercial Preparations of Antibiotic Ice

I. The procedure used employing carrageen in solution was as follows:

A 0.5% aqueous solution of carrageen (SeaKem No. 9) was prepared by sifting the carrageen slowly into water using good agitation of the water. A 300 lb. container was filled to the desired level with tap water and the contents agitated using an air bubbler. To this 500 ml. of carrageen solution was added, followed by the chlortetracycline•HCl and calcium salt. Better results are obtained if no "anti-crack" compounds are used. Agitation was continued for about ten minutes after the last material was added. Then all agitation was of necessity discontinued during the freezing cycle. When the cake was completely frozen it was removed from its container in a routine manner. Six such cakes were frozen simultaneously in a typical run. After removal from its container, the long rectangular cake was split as shown in Figure III.

The cake was sampled and analyzed at the cut surfaces as shown in Figure IV.

On the top half of the cake in the metal container was a three inch layer which was usually more opaque. This was the solution which arose to the top as the volume increased due to expansion of the freezing material. This was sampled and identified as Section D. Sample A was the innermost core, B was the main bulk of the ice and C was the surface next to the container. The concentration in each sample was determined by chipping off approximately 300 gm. of ice from the desired portion, letting the bulk of the sample melt, and then withdrawing an aliquot of the sample. The

Figure III

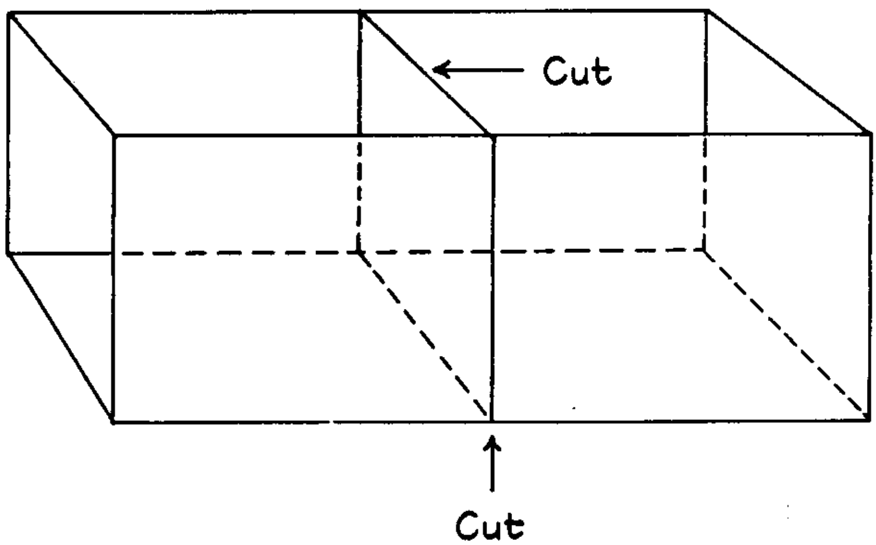
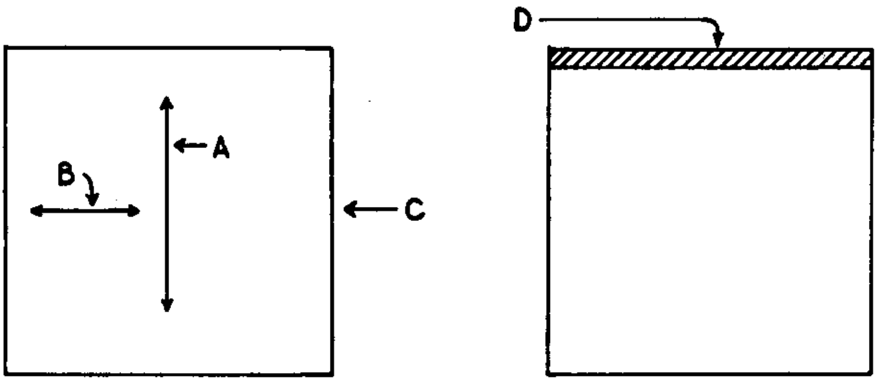


Figure IV

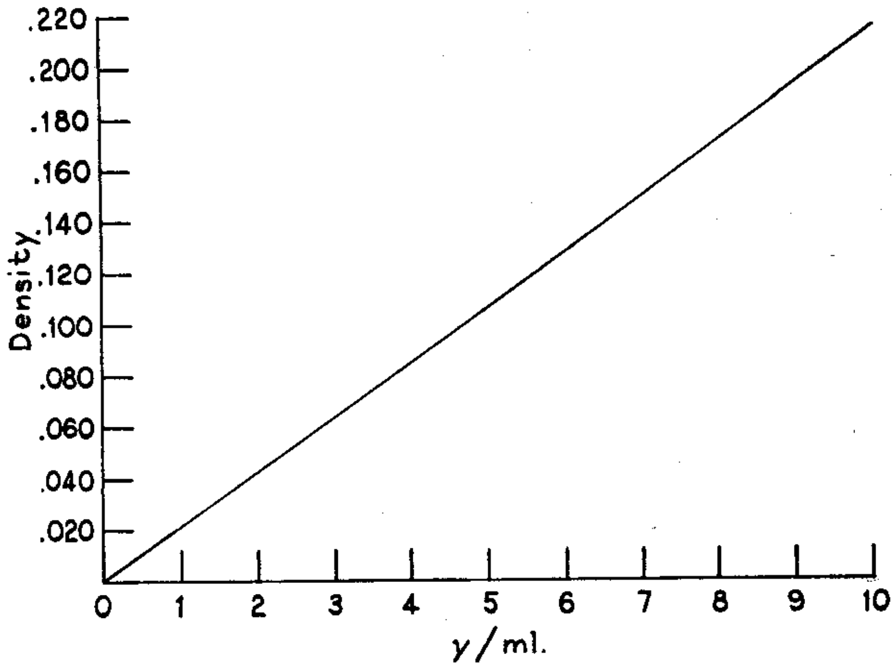


Cut Face

Side View of Top Half

aliquot was diluted with an equal volume of 0.1 N HCl. The spectrophotometric analysis was determined on the diluted aliquot using a blank prepared of composition similar to the diluted aliquot, but containing no chlortetracycline•HCl. The concentration of the sample read was determined from the predetermined concentration cure at 3700Å using a Beckman, Model DU spectrophotometer. Figure V.

Figure V
Chlortetracycline Concentration Curve



The following materials were the unit charge per 300 lb. container:

685 mg. ....Chlortetracycline HCl
(5.0 γ/ml.; 1 γ = 1 part per million)
2.5 gm. ....Carrageen
1.0 gm. ....Calcium Acetate
300 lbs. ....Water

The analyses are presented in Table II.

TABLE 2

SPECTROPHOTOMETRIC ANALYSES

Section	A	B	C	D
Cake No.	γ/ml.	γ/ml.	γ/ml.	γ/ml.
1	7.4	3.6	3.2	3.8
2	6.4	3.8	2.9	3.6
3	6.9	4.4	3.2	4.8
4	6.6	4.6	3.1	5.4
5	6.6	4.2	3.0	15.5 (?)
6	6.5	4.5	3.5	5.2

Average: 4.2 for Section B

II. The procedure using a homogeneous dry mixture of the ingredients was as follows: Three 300 lb. containers were filled from an overhead reservoir in a routine manner and placed in brine at –10°C. After air agitation had been started the following unit charge was added by slowly sifting into the water the blended powders from a standardized measure (ca. a tablespoonful):

685 mg. ....Chlortetracycline•HCl
2.5 gm. ....Carrageen
1.0 gm. ....Calcium Acetate

After the mixture had been initially agitated for fifteen minutes, this agitation was discontinued for the balance of the freezing cycle. The results and analyses of a set of cakes prepared from a dry mixture are given in Table III.

TABLE 3

SPECTROPHOTOMETRIC ANALYSES

Section Cake No.	A γ/ml.	B γ/ml.	C γ/ml.	D γ/ml.
1	6.9	2.7	3.7	6.5
2	6.4	2.7	3.5	4.8
3	6.8	3.2	3.7	4.6

Average: 2.9 for Section B

When such cakes were pulverized and the melting ice analyzed, close to theoretical values were obtained. Some slight variations occur, depending on the geographic location of the source of water.

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Un Método Prático para Dispersar Aureomycina, Chlortetracycline, en Hielo Comercial

S. D. UPHAM, F. E. STIRN, J. F. WEIDENHEIMER, F. M. CALLAHAN and L. RITTER

American Cyanamid Company Research Division
Pearl River, New York

Abstracto

Se ha encontrado un método, que tiene posibilidades comerciales, para dispersar Aureomycina en bloques de hielo de 300 libras, hecho para prevenir

deterioración bacteriológica y para extender la duración de almacenaje de pescado y otros productos alimenticios. La tendencia de ciertas soluciones de antibióticos de migrar al centro durante el congelamiento, se ha vencido con el uso de muy pequeñas cantidades de ciertos coloides protectores en combinacion con iones metálicos.

El efecto del pH, tipo de coloides protectores, así como los tipos de iones metálicos son todos interdependientes y han sido estudiados concienzudamente tanto en el laboratorio como en plantas comerciales de hielo. El método se describe en detalle y también se explican métodos para análisis.

DISCUSSION

Technology and Exploratory Fishing Session

Discussion Leader: JOHN ROBAS

Discussion Panel: WILBUR H. MILLER, GEORGE STEELE, WAYNE M. WALLER

Further Experiments On the Control of Melanosis Or Black Spot On Shrimp

M. E. BAILEY AND E. A. FIEGER

Q. Robas: Have there been any taste panel tests made on sulphite treated shrimp?

A. Bailey: Yes. We found that in the shrimp treated with 100 parts per million sulphite there was no perceptible flavor that could be attributed to the sulphite. In slightly higher concentrated ices a few individuals acutely susceptible to the sulphite taste did actually pick it up, but there is, in my opinion, no appreciable change in flavor attributable to the sulphite.

Q. Miller: I am interested in the effect of the pH of your solution on the action of the sulphite and the ascorbic acid in controlling melanosis.

A. Bailey: The pH is very important in melanin formation. The farther the pH is from the optimal for activity of the enzyme the less is the melanosis development.

Q. Miller: The stability of ascorbic acid is somewhat dependent on the pH also, and I wonder if you used sodium ascorbate, would it be more effective than the sodium bisulphite? I notice that you used 8% ascorbic acid and 92% citric acid.

A. Bailey: The primary reason that we used the 8-92 mixture was to cut down the expense. Actually, the ascorbic acid, full strength, does a wonderful job in preventing melanosis, but at the present time it is much too expensive to be