Technical Papers

Terramycin, a New Antibiotic

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A new actinomycete, *Streptomyces rimosus,* has been isolated from a soil sample and so named because of the cracked appearance of the growth on the surface of an agar medium. When the organism was grown on plates containing nutrient agar and when a variety of bacteria including certain of the Gram-negative enteric organisms, aerobic spore-formers and Gram-positive cocci were streaked across these plates, growth of the test organisms was inhibited in the vicinity of the colony of the actinomycete. When *Streptomyces rimosus* was grown under submerged aerobic conditions, the broth exhibited similar inhibitory powers, as demonstrated in serial dilution assays. From broth cultures of this organism, a crystalline antibiotic was isolated; the name Terramycin has been assigned to this compound.

Terramycin is amphoteric and forms the crystalline hydrochloride and sodium salt. Crystalline Terramycin has the following properties: mp approximately 185° C with decomposition; $[\alpha]_D^{25} - 196°$ (1.0% in 0.1 *N* HCl). It is soluble in methanol, ethanol, acetone and propylene glycol, in water to the extent of 0.25 mg per ml at 25° C; insoluble in ether and petroleum ether. Terramycin is stable over long periods in aqueous solutions at about pH 2.0–5.0, at room temperature. A sample of crystalline Terramycin analyzed: C, 53.05; H, 5.91; N, 5.64; O (by difference), 35.4.[1]

Terramycin crystallizes in several forms, depending upon the procedure used. One of these forms consists of thick hexagonal plates, the refractive indices of which are $\alpha = 1.636 \pm .004$, $\beta = 1.644 \pm .004$, $\gamma > 1.700$.

In 0.1 M phosphate buffer (pH 4.5), Terramycin shows ultraviolet absorption maxima at approximately 247, 275, and 353 mμ. It also shows characteristic absorption in the infrared region.

The activity *in vitro* of crystalline Terramycin Hydrochloride against a variety of microorganisms is shown in Table 1. The activity was determined by dissolving varying amounts of the antibiotic in nutrient agar and streaking with the organisms under test. Further observations on the sensitivity of these and other organisms will be reported in detail elsewhere.

Terramycin shows a low degree of toxicity in animals. The intravenous LD_0 for Terramycin Hydrochloride is equivalent to 103 mg of the crystalline amphoteric compound per kg of body weight in mice; the LD_{50} is equivalent to 192 mg per kg.

[1] These determinations were made by Dr. John A. Means of Chas. Pfizer & Co., Inc., Brooklyn, New York.

TABLE 1

ACTIVITY *in Vitro* OF CRYSTALLINE TERRAMYCIN HYDROCHLORIDE*

Species	μg/ml	Inhibition
Aerobacter aerogenes	1.0	100%
Klebsiella pneumoniae	3.0	"
Escherichia coli	5.0	"
Salmonella typhosa	3.0	"
S. paratyphi	1.0	"
S. schottmuelleri	1.0	"
S. pullorum	10.0	"
Shigella paradysenteriae	1.0	"
Bacillus subtilis (FDA 219)	3.0	"
Staphylococcus albus	1.0	"
S. aureus	1.0	"
Proteus sp.	> 1000	"
Pseudomonas aeruginosa	100	"
Brucella bronchisepticae	3.0	"

* Activity is expressed in terms of the equivalent weight (μg) of crystalline Terramycin necessary to inhibit growth.

As is the case with aureomycin and chloramphenicol, Terramycin is active *in vivo* as well as *in vitro* and displays marked chemotherapeutic activity against experimental infections in mice due to *Streptococcus hemolyticus, Diplococcus pneumoniae, Klebsiella pneumoniae, Salmonella typhosa,* and other organisms. It is effective by both the oral and parenteral routes of administration. Preliminary studies suggest that Terramycin has definite antirickettsial activity in the chick embryo.[2] In high concentrations it appears to inhibit the infection of the chick embryo with the PR8 strain of Influenza A virus.

[2] Data on the antirickettsial activity of Terramycin will be reported elsewhere by Dr. John C. Snyder, Harvard School of Public Health.

The Oxygenation of Blood by Gas Dispersion

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In the attempts to relieve anoxia of the tissue by means other than those of artificial respiration or inhalation of gas mixtures high in oxygen, widely varying means of extrapulmonary oxygen administration have been employed. Oxygen has been injected subcutaneously, intraperitoneally, and intravenously, as well as directly into the intestines, the joints, the renal pelvis, and the urinary bladder. Oxygen has even been applied locally in attempts to increase the absorption through the skin. All

these methods have the disadvantage of supplying only a small fraction of the total oxygen consumption of the body, and, furthermore, the intravenous injection of oxygen is limited by the inherent danger of gas embolism (*22*).

For these reasons, attempts have been made for many years to construct an apparatus that would permit the introduction of an adequate amount of oxygen into the blood by circulating it through various devices designed to expose a large surface of blood to oxygen. The oxygenators devised have consisted of one (*12*) or more (*1*, *6*) revolving disks, of chambers filled with glass beads (*4*), of spherical glass bulbs into which the blood was sprayed (*7*), of a revolving spiral sheet (*5*), and of a silk curtain through which blood was allowed to flow (*19*).

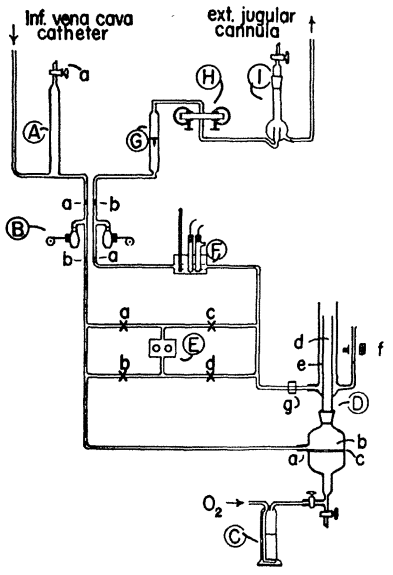

FIG. 1.

With the advent of thoracic and cardiac surgery, interest in the study of extracorporeal oxygenation of the blood was revived and efficient oxygenators consisting of one (*7–10*) or more (*15–17*) vertically revolving cylinders, a spiral tubing system (*13*, *14*), and a series of conical disks (*21*) were developed. For perfusion of the brain with oxygenated blood, an instrument having 40 to 50 rotating disks, which dip into blood flowing through a trough, was described (*2*, *3*).

In the present study, the old method of bubbling oxygen through blood (*20*), which had been discarded as too slow and foam-producing (*3*), was reinvestigated and found to be a very rapid and efficient procedure for saturation of hemoglobin under specific conditions. The oxygen is dispersed in the blood in the form of tiny bubbles produced by passing the gas through a fritted glass disk or a porcelain bacteriological filter. After oxygenation, which is nearly immediate, the excess gas is released by passing the blood over a surface coated with a methylpolysiloxane resin. The rate of oxygen flow is adjusted so as to produce optimum oxygenation without excessive flow of gas.

At the present stage of development the equipment shown in Fig. 1 is used. The dogs are anesthetized with sodium pentothal. Heparin (4 mg per kg initially and 1 mg per kg every half hour) is used as an anticoagulant. Blood pressure is recorded directly from a femoral or carotid artery, using a mercury manometer, and a pneumograph is used to record respiration rate. After filling the apparatus with fresh dog blood (about 500 ml is required) the blood is pumped from the animal through a polyethylene catheter inserted into the inferior vena cava through a femoral vein so that the tip lies near the big cardiac vein. The glass tube, A, serves to test the patency of the catheter by applying suction at A_a and also, by the use of a manometer connected to A_a, to give an indication of the reduced pressure being developed by the pump, B. The pump consists of two flexible polyethylene bottles (Plax Corporation, Hartford, Connecticut) which are alternately compressed and extended by means of a rod attached to an eccentric. Flap valves are placed at B_a and B_b.

The blood then enters the dispersion oxygenation apparatus D through a tube, D_a, fused to the glass at an angle so as to cause rapid swirling in chamber D_b. The capacity of chamber D_b is approximately 150 ml. Oxygen enters D_b through a gas-washing bottle C, containing water, which humidifies the gas and serves as an indicator of the gas flow. The gas is dispersed into tiny bubbles by the sintered glass plate, D_c, 90 mm in diam (porosity 5 μ, Corning Glass Works, Corning, New York). The gas may also be dispersed at a pressure of about 10 lb, by a porcelain filter candle (Catalog No. VFA-56, porosity 0.85 μ, Selas Corporation, Philadelphia 34, Pennsylvania). The dispersion of oxygen and blood then rises through tube D_d, which contains either fine glass rods or 3-mm glass beads coated with DC Antifoam A (Dow-Corning, Midland, Michigan). The blood then flows into chamber D_e, which has sufficient capacity to allow the remaining bubbles to escape to the surface. The level of blood is maintained at a constant level in this chamber by means of a photocell at D_f, which operates a magnetic valve at D_g.

Following oxygenation, the blood passes through a plexiglass block at F holding the large shielded Beckman pH electrodes and a thermometer. The blood circuit is arranged so as to facilitate measurement of the relative oxygen saturation of either venous or oxygenated blood by means of a modified oximeter (*15*) at E. Valves at E_{abcd} serve to by-pass a sample for the measurement of oxygen saturation. After passing through F, the blood is pumped into a rotameter, G (Size 2, Figure 27, Fischer and Porter Company, Hatboro, Pennsylvania), to give a continuous indication of flow rate, through a fine-meshed glass cloth filter, H, and into a bubble trap, I, which serves as an additional safeguard against gas emboli. The blood is returned to the animal through a cannula having a large bore (minimum 3.5 mm) inserted in an external jugular vein. All glass parts, including the glass cloth filter, but excepting the sintered glass disk, or the porce-

lain element, are coated with a silicone resin (Dri-Film No. 9987, General Electric Company, Schenectady, New York). Glass parts have also been given a permanent silicone surface by coating with a 2% solution of DC-1107 fluid (Dow-Corning Corporation, Midland, Michigan) in carbon tetrachloride, drying, and baking at 150° C for 30 min. Polyethylene tubing (0.236 in. inside diam and 0.326 in. outside diam, Surprenant Manufacturing Company, Boston, Massachusetts) is used throughout. Satisfactory connections between polyethylene tubing and glass tubing can be made by *carefully warming* the end of the plastic tubing over an open flame before sliding it over the glass. Before use, the apparatus is thoroughly washed by the circulation of Dakin's solution and sterile saline. The entire equipment is mounted in a plexiglass cabinet equipped with a device to circulate air at about 40° C.

TABLE 1

Dog No.	Weight	Mean blood flow	Change in mean arterial blood pressure	Change in heart rate	Change in respiratory rate	Duration of nitrogen inhalation
	kg	ml/min	mm Hg	%	%	min
1	10.0	300	− 5	+21	+ 37	7
2	16.0	500	−15	+ 8	+ 22	12
3	12.0	500	−30	0	0	20
4	12.0	200	−40	+ 9	+ 20	25
5	10.0	320	−50	+12	+ 33	58
6	10.0	500	−33	+21	+ 83	15
7	12.0	500	−15	0	− 20	32
8	14.4	600	...	...	+ 10	26
9	11.7	450	− 3	0	− 8	99
10	9.0	530	...	...	0	30
11	11.7	460	−10	+10	+ 6	60
12	6.8	350	− 5	0	+143	28
13	9.1	350	−16	0	+ 87	60
14	9.1	250	0	0	0	8
15	9.0	500	− 2	0	− 11	90

In order to test the efficiency of the oxygenation apparatus, the dogs were allowed to breathe 100% nitrogen through a tight-fitting transparent plastic mask. Pumping of oxygenated blood preceded the administration of nitrogen by 5 to 10 min. During the period of pumping, hemolysis increased slowly and occasionally reached values of 0.4 g/100 ml of plasma. There occurred also a drop in leukocyte count, owing mainly to a destruction of granulated cells.

Table 1 shows the periods of nitrogen inhalation that 15 consecutive dogs survived while the blood was circulated through the oxygenator at the rate indicated. In dog No. 9, the period of nitrogen inhalation was extended for 99 min. The acute rise in blood pressure, always associated with acute anoxic anoxia, was completely prevented. In most instances the heart rate and respiratory rate showed moderate increases. Electrocardiograms taken during the inhalation of nitrogen were compared with the tracings before and after the experiment and failed to show any signs of general anoxemia, like diminutions of the T and R wave or depression of the S-T segment.

The high degree of oxygen saturation of the blood leaving the apparatus was always apparent, since the striking bright red color stood in great contrast to that

TABLE 2

Source of blood	28-Min period of nitrogen breathing		88-Min period of nitrogen breathing	
	O_2 saturation %	pH	O_2 saturation %	pH
Venous blood to apparatus	58.8	7.32	63.1	7.15
Oxygenated blood from apparatus ...	98.0	7.38	...	...
Carotid artery	83.2	7.30	97.1	7.00

dark blood entering the apparatus from the vein, and even to that visible in the carotid cannula, used to record blood pressure. The oximeter readings have consistently indicated the saturation to be above 95% and the Van Slyke analyses, reported in Table 2, for a typical experiment reveal an entirely satisfactory saturation of the blood in the carotid artery. These samples were collected *while the dog was breathing 100% nitrogen.* The constancy of the blood pH indicates that carbon dioxide was released at the same rate oxygen was absorbed.

If the pump is stopped during the inhalation of nitrogen, respiratory arrest occurs within 40 sec.

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