RESUME OF LITERATURE :

The phenomenon of antagonism amongst micro-organisms was known to microbiologists much earlier than the actual introduction of the metabolic products of such micro-organisms in therapeutics. As early as 1877, Pasteur and Joubert (Wallace, 1970) noted inhibition of anthrax bacillus by air borne micro-organisms. They have even suggested that this phenomenon of antibiosis might be useful in the treatment of certain infections. It was subsequently established that these antagonistic micro-organisms elaborate metabolic products, which are toxic to microbes and have specific chemical and biological properties.

Vuillemin (1889) coined the phrase 'antibiosis' and defined it as 'a type of association, in which one living creature destroys another in order to sustain its own life'. Later Waksman (1947) coined the noun 'antibiotic' from this phrase (antibiosis). He defined an antibiotic as a chemical entity elaborated by a micro-organism as a metabolic product in the course of its growth.

The ability of soil micro-organisms to survive rigorous exposure to fatal conditions of weather and mutual competition amongst themselves for survival, prompted large number of microbiologists to study them for antibacterial activity. Detailed studies of these soil micro-organisms revealed the presence of several actinomycetes which possessed antibacterial activity. Observations of cultures isolated by Gasperini (1892-1895), Muller (1908) Greig-Smith (1911-1917), Lieske(1921), Gratia and Detli (1924-1927), and Rosenthal (1925) demonstrated that these organisms produce chemical substances which inhibit the growth of other micro--organisms. The first substances to be isolated in this manner were mycophenolic acid (Gosio, 1896), which inhibited Bacillus anthracis and pyocyanin derived from Pseudomonas aeruginosa (Emmerich and Loew, 1899). Severe toxic reactions to these substances precluded their use in chemotherapy (Waksman, 1947a).

Stimulated by the work of Greig-Smith(1917) who demonstrated antagonism amongst actinomycetes and bacteria, Welsch (1937) isolated actinomycetin from a streptomycete culture. This chemical substance possessed interesting properties but could not be utilized because of its toxic reactions.

Dubos (1939) subsequently discovered and isolated gramicidin and tyrocidin from the spore bearing bacillus B. brevis. These two chemical substances obtained as metabolites were probably the first compounds to be used in the chemotherapy by topical application.

The second world war imposed conditions which necessitated search for new therapeutic agents specific for the treatment of systemic diseases of microbial origin. Florey and Chain (Florey 1949) isolated penicillin from the mould penicillium based on the observations of Alexander Fleming (1929). The discovery of penicillin revolutionized the entire field of therapy and it was demonstrated for the first time, that, a potent chemical compound almost free from toxic reactions could be obtained from micro-organisms for antibacterial chemotherapy (Abraham et al.1941). The manufacture of penicillin revolutionized the approach to various problems in the field of antibiotic research and indirectly intesified the search for new antibiotics.

Introduction of streptomycin (Schatz et al. 1944) was a second landmark in the field of antibiotics. Its effectiveness in tuberculosis, an age

old disease of humans, came as a great boon. It is evident from the fact that though streptomycin is relatively more toxic than penicillin it has been accepted more readily with eagerness and has retained its position of importance till today.

The efficacy of streptomycin against gram--positive, gram-negative and acid fast bacilli led to the introduction of broad spectrum antibiotics. The toxic nature of the substance prompted further work and introduction of newer antibiotics, like neomycin (Waksman and Lechevalier,1949), viomycin (Bartz et al.1951; Finlay et al.1951) kanamycin (Umezawa et al.1957) and paromomycin (Coffey et al. 1959). These antibiotics represent a class of chemical compounds produced by Streptomyces species and are designated as "basic antibiotics of streptomycin group'.

The antibiotics of the streptomycin group represent a class of chemical substances obtained as metabolites from micro-organisms (actinomycetes). These substances namely streptomycin, kanamycin, viomycin, neomycin and paromomycin differ amongst themselves but possess many similar chemical and biological properties. They were primarily developed as antitubercular agents. However, only strepto-

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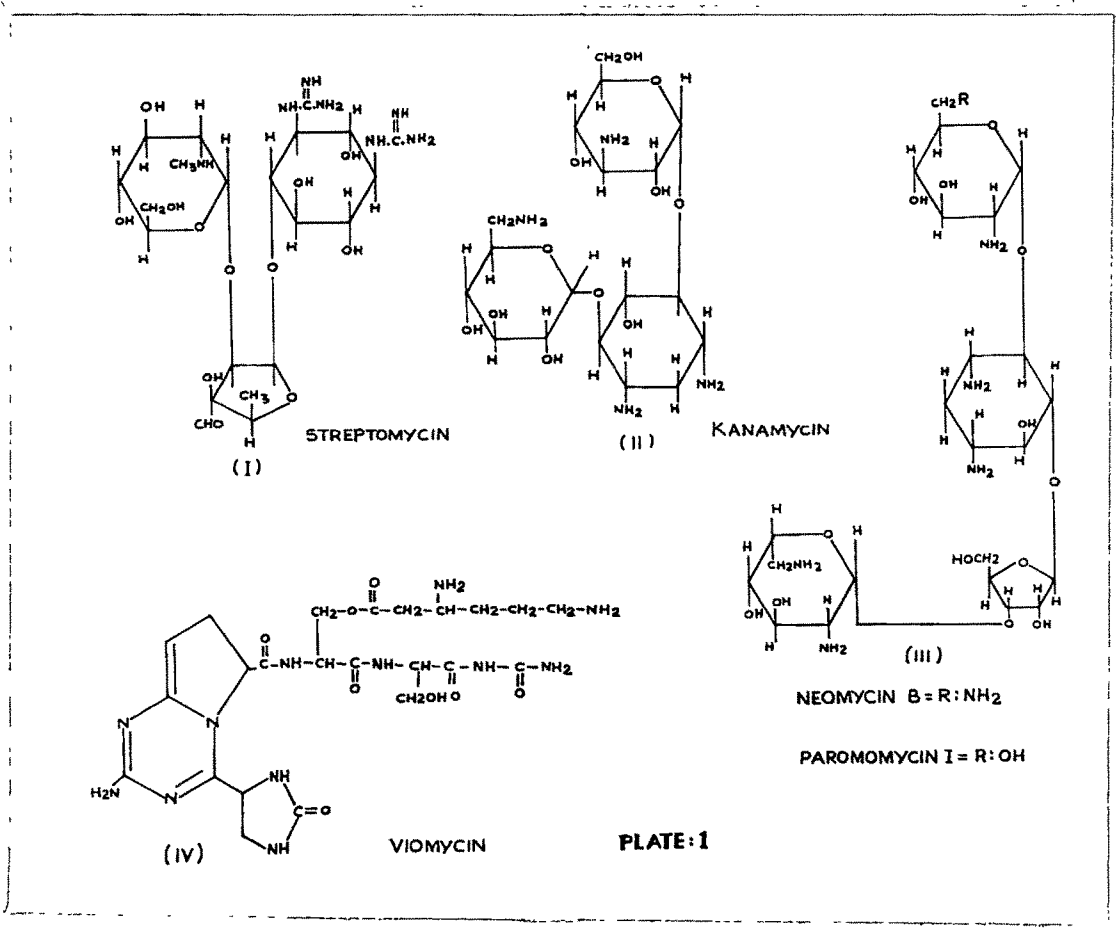
Plate No.1



Note: Structure (III) is general formula for neomycin B and paromomycin I. Structure of viomycin (IV) is as per Dyer et al.(1964).

mycin has been advocated as the drug of first priority for this specific action. Neomycin and paromomycin are preferred for their therapeutic action on infections of gastro-intestinal tract, while kanamycin and viomycin are preferred for systemic use only in the cases where streptomycin resistant organisms are encountered with.

Chemistry

The streptomycin group of antibiotics give positive Sakaguchi reaction indicating a guanido group which forms the central core of the molecule. This central core is attached to two sugars by glycosidic linkages and one of these two sugars is an aminohexose (Evans, 1965), the exception being viomycin (Haskell et al. 1952) which is a polypeptide. The structural formulae and the relationship of different units to each other in the molecule of these antibiotics posed a formidable problem to organic chemists. However, degradation studies on these antibiotics revealed the chemical configuration and stereochemistry of different moieties of the antibiotics. (Plate 1).

Streptomycin isolated from streptomyces griseus (Schatz et al. 1944) was obtained as pure

crystalline reineckate (Fried and Wintersteiner, 1945) for purposes of analytical determinations and as helianthate (Kuehl et al. 1945) for conversion into hydrochloride or sulphate salts. Streptomycin molecule is composed of three substances streptidine, streptose and N-methly-L-glucosamine. A hypothetical reaction of these three substances takes place biogenetically with the elimination of two molecules of water to yield streptomycin (Brink and Folkers, 1949). The glycosidic linkage between streptidine and streptose moiety of streptomycin is weaker and can be easily cleaved whereas the linkage between streptose and N-methyl-L-glucosamine is much stronger and rather difficult to break. Consequently, by mild acid hydrolysis of streptomycin, streptidine and glycosidically linked streptose and N-methyl-L-glucosamine were obtained. This disaccharide was designated as streptobiosamine (Kuehl et al. 1946).

Aqueous acid hydrolysis yields diacidic base streptidine (Peck et al. 1945). Stepwise alkaline hydrolysis of streptidine gives strepturea and then a diamino compound streptamine. Carter et al.(1946) and Peck et al. (1946) subsequently proved that the guanido groups in streptidine are placed in 1,3 position. The structure of strepti-

dine as 1, 3-diguanido-2, 4, 5, 6-tetrahydroxy cyclohexane was confirmed and its stereochemical configuration elucidated by synthesis from streptamine (Wolfram and Olin, 1948).

Alkaline hydrolysis of streptidine by lithium hydroxide for a prolonged period (48 hours) produced ammonia and a diamine streptamine. The entire nitrogen in streptamine was titrable by Van Slyke reaction, indicating the existence of nitrogen as amino nitrogen. The lack of optical activity indicated that streptamine has meso configuration and confirmation of its structure and stereochemistry was provided by synthesis from D-glucosamine (Wolfram et al. 1959).

Umezawa et al. (1957) described the antibiotic kanamycin produced by an actinomycete which was designated as K2J - later identified as Streptomyces kanamyceticus nsp. Paper chromatography of fermentation broth gave three spots which showed antibiotic activity (Cron et al.1958; Gourewitch et al. 1958; Schmitz et al. 1958 and Umezawa et al. 1958). One of the antibiotics was found to be toxic. These three antibiotics were later named as kanamycin A, B and C.

Kanamycin A, the major component of the fermentation broth was more resistant to acid and alkaline hydrolysis but on refluxing with 6-N hydrochloric acid, yielded 2-deoxystreptamine (Maeda et al. 1957; and Cron et al. 1958, 1958a) and two aminosugars identified as 6-deoxy-6-amino--D-glucose and 3-deoxy-3-amino-D-glucose (Cron et al. 1958a, 1958b). The hydrolysis of fully methylated kanamycin A afforded 5-methyl ether of 2-deoxystreptamine. Survival of the deoxystreptamine unit when kanamycin A was oxidized by periodate confirmed that the glycosidic linkages were at C-4 and C-6 positions (Cron et al. 1958b). The infrared spectrum of kanamycin A was indicative of the α-glycosidic linkages and the strong dextro rotation of the deca-acetate supported the view (Cron et al. 1958c).

Another antitubercular antibiotic designated as viomycin was isolated from Streptomyces floridae, Streptomyces puniceus and Streptomyces vinaceus in 1951 independently by Bartz et al. (1951) and Finley et al. (1951).

Haskell et al. (1952) in their studies on structural configuration of viomycin have shown that viomycin is a polypeptide with basic proper-

ties. The biuret, ninhydrin and Sakaguchi (for guanidine group) tests were positive. Acid hydrolysis products of viomycin have been shown to contain L-serine, L-α-β-diaminopropionic acid and L-β-lysine. In addition to amino acids, carbon dioxide, ammonia and urea have also been found. A guanido compound was also shown by Dyer et al. (1964) which they designated as viomycidine and identified as 3-guanido-1-pyrolino-2-carboxylic acid. Bowie et al. (1964) confirmed the presence of viomycidine at a later stage and postulated that viomycidine occurs in the molecule of viomycin, in a cyclic form as a pyrimidine (Bowie et al.1964a)

Hydrolysis of peptide-A obtained from partial hydrolysis of viomycin by boiling with 12-N-hydrochloric acid gave α-β-diaminopropionic acid and viomycidine in equimolecular ratio. This led Bowie et al. (1964a) to postulate the structure of peptide-A and to prove that chromaphore of peptide-A is also the chromaphore of viomycin. Further hydrolysis of viomycin yielded peptide-B as well as two more peptides-C and -D.

The analysis of peptide fragments and their hydrolysis products led Bowie et al. (1964a) to propose structure of viomycin. This was later

denied by Dyer et al. (1964) who proposed an alternate structure.

Neomycin is the collective name for several antibiotics elaborated by a strain of Streptomyces fradiae isolated by Waksman and Lechevalier (1949). Various procedures for separation and purification of these different fractions are reported (Peck et al. 1949; Leach de Vries et al.1951 and Swart et al. 1951). Counter current distribution method has shown that the neomycin complex consist of neomycins A, B and C. The antimicrobial activity of the mixture was comparable to that of streptomycin.

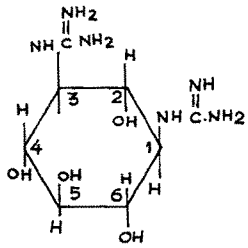
Treatment of neomycin B and C by methanolic hydrogen chloride gave identicale amprophous amine hydrochloride and non-identicale methyl glycosides (methyl neobiosamine B and C.). The amorphous amine designated as neamine was isolated and found to be identicle with neomycin A (Leach et al. 1951 and Leach and Teeters, 1951). Dutcher and Donin (1952) confirmed the finding. Hydrolysis of neamine gave deoxystreptamine with the structure 1,3-diamino-4, 5,6-trihydroxy cyclohexane (Carter et al. 1961).

Rinehart et al. (1958-1960) established the

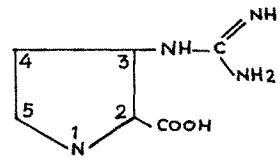
structure of neobiosamine B and C. Neobiosamine B is a disaccharide composed of diaminohexose (neosamine B) and D-ribose linked glycosidically. Neobiosamine C is a disaccharide composed of 2,6-diamino-2,6-dideoxyhexose with the configurationof D-glucose (neosamine C) and D-ribose also linked glycosidically. Neomycin B and C are isomers being bound with hydroxyl group at C-3 of ribose. The commercial preparation of neomycin consist mainly (90 per cent of the bulk) of neomycin B.

Paromomycin was isolated in the year 1959 by Coffey et al. from a strain of Streptomyces rimosus paromomycina. Degradation of paromomycin by methanolic hydrogen chloride gave paromamine which was isolated as crystalline hydrochloride. Vigorous acid hydrolysis of this product yielded an optically inactive compound identical with deoxystreptamine that is 1,3-diamino-4, 5,6-trihydroxy cyclohexane. Less vigorous hydrolysis yielded deoxystreptamine and sugar, subsequently identified as D-glycosamine (Haskell et al. 1959).

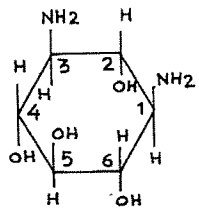
The second component isolated was paromobiosamine a disaccharide: O-(diaminohexosyl)-D-ribose. One of the sugars in the molecule of parmomobiosamine was designated as paramose and the other as



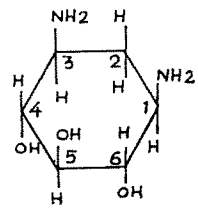
STREPTIDINE



VIOMYCIDINE



STREPTAMINE



2- DEOXYSTREPTAMINE

PLATE 2

D-ribose. Haskell et al.(1959) proposed the complete structural formula of paromomycin. Paromomycin contains in addition, a minor component called paromomycin II (Horii et al. 1963).

Rinehart et al. (1962) have shown structural similarities between neomycin, kanamycin and paromomycin since all contain 2-deoxystreptamine. Streptomycin contains a diguanido moiety designated as streptidine while viomycin contain guanido group with different linkages (Haskell et al.1952). The absolute stereochemistry of streptamine has been shown to be analogous with 2-deoxystreptamine (Dyer and Todd, 1963) (Plate 2).The central core of these antibiotics is linked glycosidically with sugar moieties which are also interlinked glycosidically. Viomycin which is a polypeptide antibiotic is the only exception containing guanido group but not the sugar residues (Haskell et al. 1952).

These antibiotics are produced by streptomyces group of micro-organisms. Studies with tracer compounds labelled with C^{14} have shown that the complete skeleton of streptomycin is synthesized from D-glucose while acetate is mainly incorporated in guanidyl side chain (Hunter, 1956). That is why

as per the classification proposed by Hunter (1956) these compounds are designated as 'antibiotics derivable from sugars'. In terms of mode of action of these antibiotics, they are also called 'streptomycin group' of antibiotics as they have almost similar mechanism of action.

Pharmacology

The similarities in the chemical structure and stereochemistry of these antibiotics are well reflected in their biological activities. However, no reports are available which correlate these structural similarities with the biological actions. The major pharmacological profiles available concern absorption, distribution, exgretion and toxic reactions. The severe toxic reactions exhibited by these antibiotics have interested a large number of investigators. The neuromuscular blocking action of these antibiotics has received major attention. The remarkable similarity in the biological actions of these antibiotics though produced by different organisms, is interesting.

None of these antibiotics are absorbed when administered orally. Hence, parenteral administration is utilised to attain clinically useful blood

levels and systemic antimicrobial action thereof. Poor absorption from the alimentary tract makes them useful in the treatment of infections of the intestinal tract. Their broad spectrum antimicrobial activityis ideally suited for this action.

Therapeutic blood levels are easily obtained on intramuscular injection. Streptomycin, kanamycin and viomycin attain peak levels in about 2 to 4 hr and the levels are well maintained for about six to eight hr (Heilman et al. 1945; Zintel et al. 1945; Marshall, 1948; Warmer et al. 1951; and Welch et al. 1958). Due to relatively high nephrotoxicity such data about neomycin is not available, though experiments in dogs reported by Spencer et al. (1950) demonstrated that neomycin is also easily absorbed by parenteral route. These antibiotics are excreted in the urine without undergoing any metabolic change or loss of antibacterial activity.

Acute streptomycin poisoning produces restlessness, laboured respiration, loss of consciousness and coma. In cats and dogs, nausea, vomiting and ataxia may also be observed (Molitor and Kuna, 1949). Similar toxic reactions were observed with neomycin, kanamycin (Tisch et al.1958) and viomycin (Brazil et al.1961). Coffey et al.

(1959) reported moderate kidney damage due to paromomycin toxicity.

These antibiotics did not have any effect on the blood pressure of anaesthetized rabbit, cat and dog in low doses (10-20 mg/kg) (Molitor et al. 1946; Corrado, 1958; Tisch, et al. 1958; Veis, et al. 1963 and Jeske et al. 1964). However, in higher doses (200-400 mg/kg) they lowered the blood pressure to 10-15 mm Hg. Molitor et al.(1946) demonstrated that with this doses, the blood pressure of anaesthetized cat did not show any recovery though the heart continued to beat normally and the animal survived if maintained on artificial ventilation.

Streptomycin (77 mg/kg) was shown to inhibit salivation in anaesthetized cats. Recovery occured gradually to about 50 per cent (Corrado, 1958). Neostigmine not only reestablished the salivation but made it more pronounced than control salivation. This dose of streptomycin was also shown to relax the contracted nictitating membrane, block vagal action on the heart, decrease the release of sympathin on splanchnic stimulation and convert the depressor response to pilocarpine to a biphasic response. All these effects of streptomycin

were reversed by calcium (Corrado, 1958). Zaijabov (1961) reported that streptomycin produced predominence of sympathetic system. Ganglion blocking action was reported for viomycin (Brazil et al. 1961); kanamycin (Corrado and Ramos, 1960), and neomycin (Ramos et al. 1962).

The effect of autonomic drugs like acetylcholine, adrenaline and noradrenaline on blood pressure of anaesthetized animals is not modified following these antibiotics. Tisch et al. (1958) reported that kanamycin slightly inhibited the blood pressure response to acetylcholine while that to adrenaline or noradrenaline was potentiated. However, this changes was not significant. Streptomycin was shown to suppress peristaltic activity of guinea pig ileum and exhibit anti-acetylcholine (Dzoljie and Antaneoekovic, 1965) and antihistamine (Leaders et al. 1960; Popovici et al.1965) actions also. The effect of streptomycin on uterine musculature was shown to be stimulant in low doses, is blocked by atropine and was attributed to cholinergic action, while the depressant action in high doses was attributed to direct musculotropic action (Popovici et al. 1967).

Streptomycin was shown to depress the

isolated frog and rabbit hearts (Leaders et al. 1960; Gomazkov, 1963). It produced atrioventricular block, while neomycin manifested negative inotropic action. However, streptomycin (1-2 g) fail to produce cardiac depression in heart lung preparation of dog (Swain et al. 1956). Streptomycin depressed the cholinestrase activity of the rabbit (Masayasu, 1954 and Hirata 1964) and the human serum.

These antibiotics as a group exert severe toxic reaction on the 8th cranial nerve producing ototoxicity i.e. cochlear and vestibular manifestations. The 8th cranial nerve toxicity of streptomycin (Molitor and Graessle,1950) served as a handicap in the long term treatment of tuberculosis. Kanamycin was less toxic than streptomycin and neomycin in this respect (Owada, 1962 and Oka,1965). Prolonged administration of viomycin also produced disturbances of co-ordination and hearing loss (Vies et al.1963; Veis and Bykova, 1965) but is less severe as compared to streptomycin. The ototoxicity produced by neomycin and kanamycin was shown to be persumabely different from that produced by streptomycin (Vermier and Alleva, 1960).

Investigating the neurotoxicity of kanamycin, Owada (1962) used the degradation product of kanamycin (2-deoxystreptamine) to find the molecular moiety responsible for the toxic reaction. His findings indicated that 2-deoxystreptamine had the strongest toxic reactions in mice, while glucosamine showed maximum ototoxicity in guinea pigs. The findings also indicated that the 8th cranial nerve toxicity of this antibiotics was the function of the molecule as a whole rather than separate moieties of the molecule.

Reduced toxicity on 8th cranial nerve with kanamycin and viomycin is obliterated by increased nephrotoxicity. Thus in terms of clinical usefulness streptomycin still remains the antibiotic of choice for long term treatment in tuberculosis and other diseases.

The neuromuscular blocking action of these antibiotics is by far the most extensively studied pharmacological activity. Attention to this serious toxic manifestation was drawn by clinicians (Hinshaw and Feldman, 1945; Pridgen, 1956; Engel and Denson, 1957; Middleton, 1957 and Webber, 1957) who observed respiratory distress and paralysis of respiratory muscles in patients maintained on high

doses of streptomycin undergoing surgical treatment. Deaths due to intraperitoneal infiltration of neomycin in patients under anaesthesia were also reported.

The correlation of neuromuscular blocking action with respiratory distress and paralysis was first demonstrated by Brazil and Corrado (1957) with streptomycin. They also demonstrated that calcium and neostigmine abolished this block and relieved respiratory distress. Jindal and Deshpande (1958) confirmed these findings and further demonstrated that neostigmine failed to relieve this block when it was complete. They also pointed out that no correlation could be established between the neuromuscular block and the toxic reaction of these antibiotics on the 8th cranial nerve i.e. ototoxicity.

The neuromuscular blocking action of streptomycin and neomycin was potentiated by anaesthetics like ether (Pittinger and Long, 1958; Corrado et al. 1959 and Osterloh, 1963) and muscle relaxants like d-tubocurarine (Timmerman et al. 1959; Bezzi and Gessa, 1961). The neuromuscular blocking action of kanamycin (Corrado and Ramos, 1960; Goldberg, 1964), viomycin (Adamson et al. 1960 and

Brazil et al. 1961) and paromomycin was likewise demonstrated (Adamson et al. 1961).

Characterizing the blocking action of streptomycin as similar to magnesium ions, Brazil and Corrado (1957) pointed out that calcium and neostigmine abolished this block. In his later work Brazil (1960) also showed that in denerveted tibialis anticus muscle of the dog, streptomycin depressed the muscle and calcium increased the depression. The contractile response to potassium chloride and acetylcholine was temporarily abolished by streptomycin. He characterized this anti-acetylcholine action as due to stabilizing action of streptomycin on the skeletal muscle fibers. In a similar work Speranskaya (1964) showed that the neuromuscular blocking action of streptomycin was blocked by cysteine.

Iwatsuki et al. (1958) demonstrated that streptomycin antagonized the action of succinylcholine and decamethonium, whereas it potentiated that of d-tubocurarine. They proposed a competitive inhibition of acetylcholine by streptomycin at myoneural junction. A similar mechanism of action was also proposed for kanamycin by these workers. Neomycin had different mechanism of action when used

alone. Streptomycin (40 mg/kg) reduced the dose of d-tubocurarine in rabbit head drop and this action of streptomycin lasted for about 24 hr (Sikh and Sachdev 1965).

Brazil, Corrado and Berti (1959) investigated the molecular structure responsible for neuromuscular blocking action of streptomycin. They used streptomycin and its degradation products namely streptidine and streptamine. These three compounds possessed neuromuscular blocking action on cat gastrocnemius preparation. The action of streptidine was very similar to d-tubocurarine while that of streptamine and streptomycin was similar to each other. Thus it was apparent that the degradation products of streptomycin also possessed neuromuscular blocking action. Investigating the action of streptomycin on frog nerve *in vitro*, Sokoll and Dieke (1969) showed that the nerve toxicity was mainly produced by decrease in conduction velocity due to change in the sodium permeability.

Comparative data indicated that neomycin was most potent neuromuscular blocking compound amongst these antibiotics (Ramos *et al.* 1962), while the potencies of streptomycin, kanamycin and

viomycin were in decreasing order (Kubikowski and Szreniowski, 1963). The block produced by neomycin, streptomycin and viomycin was antagonized by calcium and neostigmine while that due to kanamycin was potentiated by neostigmine (Timmerman et al. 1959; Osterloh, 1961). Naiman et al. (1965) reported that calcium antagonised the neuromuscular block produced by kanamycin more effectively. The block due to neomycin, streptomycin and viomycin was found to persist for longer periods while that due to kanamycin was observed for shorter duration (Corrado and Ramos, 1960).

According to the findings of Elmquist and Josefesson (1962), neomycin reduces the release of the neurotransmitter acetylcholine in response to stimulation of the motor nerves. They also showed that neomycin reduces the amplitude of end-plate-potential. This action of neomycin was antagonized by calcium ions. This finding was further confirmed by Brazil and Franceschi (1969) who demonstrated decreased release of acetylcholine in rat phrenic nerve diaphragm preparation.