Presumptive identification of aminoglycoside antibiotics by the pH susceptibility disc agar-diffusion method

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Summary. Using the pH (buffered) sensitivity discs for the agar-diffusion bioassay of aminoglycoside antibiotics, characteristic response curves were obtained. Since the nature of the activities observed are structure-related, this method can serve as a useful aid for primary identification of members of this class of antibiotics.

Many aminoglycosides and aminocyclitols are useful, broad-spectrum antimicrobial agents[1-6]. The continued search for newer ones with better pharmacologic properties, requires an early decision about the possible novelty of the new entity. Once the separation from other antibiotic groups with basic character, like macrolides[7,8], and certain protein and peptide antibiotics has been made, the closer identification can be facilitated by the quick biologic method described below.

Materials and method. The aminoglycoside antibiotics included in these experiments were: amikacin (semisynthetic), streptomycin, tobramycin, kanamycin, gentamicin, neomycin, neomycin B, butirosin, paromomycin, ribostamycin, sisomicin, lividomycin, hygromycin B, spectinomycin (aminocyclitol), and kasugamycin. They were used as salts or free bases dissolved in deionized water.

The method employed utilizes the principle of classification of antibiotics by means of the pH-chromatograms[9]. In place of strips of chromatographic papers impregnated with buffers of various pH-values, we used antibiotic susceptibility discs. Sensitivity discs (6.35 mm diameter) were pretreated with McIlvaine's citric acid-phosphate buffers[10] (pH between 2.3 and 8) and with phosphate buffers (pH 9 and 10), and stored in dry condition. McIlvaine's buffer is especially convenient for assay of crude preparations since the citric acid component tends to bind cations that interfere with the assay. Discs were individually saturated prior to the test with the appropriate aqueous antibiotic solutions to contain 10 µg of the compound. They were placed on the surface of Penassay agar plates seeded with spores of *Bacillus subtilis* ATCC 6633, and the plates were incubated overnight at 30 °C. The diameters of the inhibition zones were measured in mm. Duplicate experiments were carried out with freshly prepared solutions using 2 discs for each antibiotic in each experiment. The results presented are the mean of the 4 values from these experiments. In all cases, the zone diameters of the replicates were within 1–2 mm of each other depending on the purity of extracts.

Results and discussion. The diameters of the inhibition zones for each antibiotic were plotted and the corresponding curves are delineated in figures 1 and 2. Characteristically, the diameters of inhibition zones are small at the more acidic pH values (pH 2.3–4), increase at pH 5, and reach high values at pH 7. Further increase of zones at pH values of 8–10 is moderate. Kasugamycin is an exception to this finding. The potency curves show that the kind and

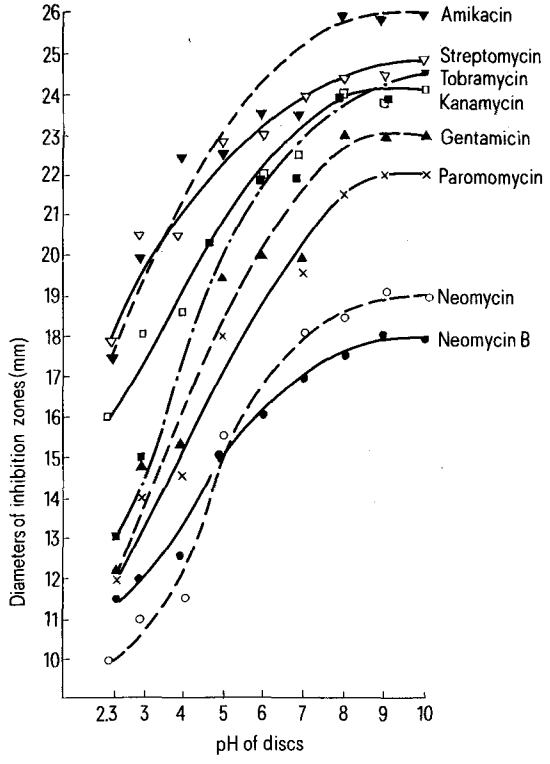

Fig. 1. Effect of pH on the inhibition zones obtained with some commonly-used aminoglycoside antibiotics (10 µg/disc) against *B. subtilis* ATCC 6633.

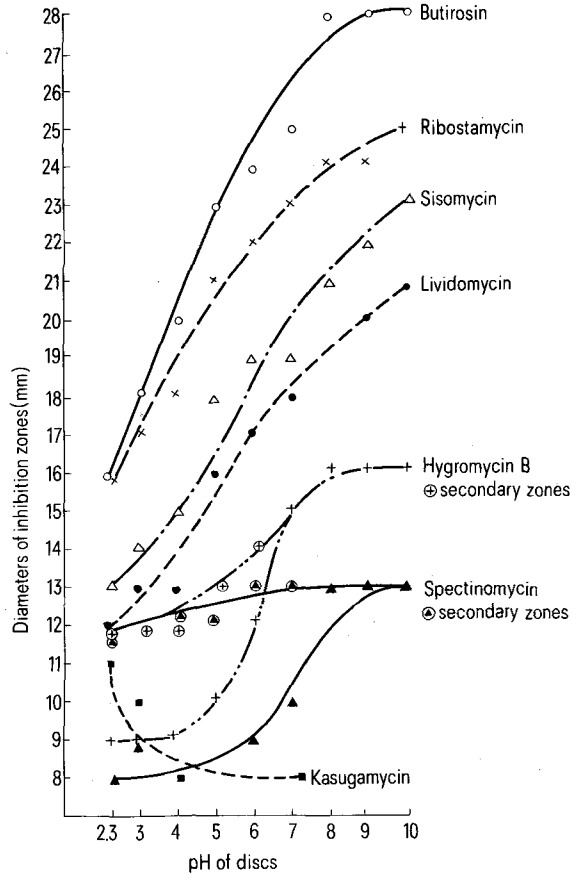

Fig. 2. Effect of pH on the inhibition zones obtained with more additional aminoglycoside antibiotics (10 µg/disc) against *B. subtilis* ATCC 6633.

amount of activities appear to be structure-related within the broad class of aminoglycoside antibiotics.

As for the kind of activity, the following features of the potency curves could serve as a useful aid for identification: a) shape of the curve, b) steepness of the middle part of the curve, c) the appearance of the upper and/or lower parts of the curves, d) double inhibition zones at the acidic pH values which merge at around the neutral pH (hygromycin B and spectinomycin), e) inhibition zones only at certain pH values (kasugamycin). For the amount of activity the following parameters are to be considered: 1. absolute zone sizes at the various pH values and 2. eventual secondary zones.

The diffusibility and potency of the individual aminoglycoside antibiotics at different pH-values seem to allow for the use in a simple, rapid and relatively reproducible assay for the early screening and identification of most of the entities in this group of antibiotics. For example, we have been able to identify neomycin one of the most frequently occurring aminoglycoside antibiotics in the early phase of isolation by this method. The pH activity curve of neomycin and that of a number of unknown isolates later shown by additional biological and chemical tests to be neomycin were almost superimposable. It is expected that the case is similar with the majority of other aminoglycosides.

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Certain biochemical changes in the ovary of a migratory bird, *Calandrella acutirostris tibetana*

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Summary. The changes in the ovarian cholesterol, glycogen and ascorbic acid of Bagheri, *Calandrella acutirostris tibetana,* during its non-breeding and breeding periods were studied. There was a significant increase in all the 3 biochemical constituents during the breeding period.

The changes in the chemical composition of the different tissues during different seasons in tropical nonmigratory birds are well documented. There is, however, practically complete lack of information on the altered chemical composition of different tissues during different seasons of a tropical migratory bird. Therefore, it was considered of interest to study the changes in the level of cholesterol, glycogen and ascorbic acid in the ovary during non-breeding and breeding periods of a tropical migratory bird, Bagheri, *Calandrella acutirostris tibetana.*

Materials and methods. Bagheri, *Calandrella acutirostris tibetana* is a migratory, small-sized sparrow-like bird available in plenty in Patna 25.37 N 85.12 E and its adjoining areas from September to April; it then migrates to Ladakh 34.30 N 77.30 E for breeding from May to July (Ali and Dillon[2]). In order to study the complete cycle of reproduction, a good stock of birds was kept in large aviary cages in April and maintained till July for monthly sampling. The cages were kept in a well ventilated and lighted place and temperature was maintained at 14–16 °C. The birds in captivity showed migratory restlessness. The birds were fed ad libitum on a nutritionally adequate diet, and were weighed at regular intervals. Fresh water was always available. Rigorous efforts were made to assure that the birds were disturbed to a minimum extent.

The birds weighing 22–24 g were taken and ovary was dissected out and accurately weighed. The routine histological investigations for the complete ovarian and thyroidal cycles were made throughout the year. Ovarian cholesterol was estimated by Sackett's method as described by Varley[3]. The glycogen was estimated according to the method of Kemp and Andrienne[4]. The ascorbic acid concentration was determined according to the 2-4 dinitrophenyl-hydrazine method of Roe[5].

Results and discussion. The ovary was in a regressed state during the non-breeding period (August–mid April). A noticeable increase was evident in the last week of April and this weight change continued till July. Ovarian weight increased 42-fold from April to July, and this increase was exponential. The development of the ovarian follicle was intense but brief. Mature ova were observed but no post-ovulatory follicles were observed in the breeding period (May to July). However, it was interesting to note that in the male, sperm were present in the lumen of the tubule in June during the breeding period in captivity.

The concentration of cholesterol, glycogen and ascorbic acid in the ovary showed a significant increase during the breeding period (table). Since cholesterol is a precursor of steroid hormones, it was expected that cholesterol content of the ovary would increase due to the greater demand for cholesterol for the production of ovarian hormones like estrogen and progesterone. The synthesis of estrogen in a migratory bird is important since estrogen is concerned with the increase of migratory fat that accumulates in the s.c. and i.p. areas (Thayer et al.[6]). Thyroid hormone plays an important role in controlling the plasma cholesterol level. It has been established that thyroid hormone depresses plasma cholesterol as well as liver cholesterol level (Turner et al.[7]; Handler[8]). If so, plasma cholesterol level should be higher when the thyroid activity was less. The annual gonadal cycle in most of the finches studied in tropical birds was inversely related to thyroid activity cycle. Gonads were at peak-phase of activity when thyroid function was lowest and vice-versa (Thapliyal[9,10]). Besides, surgical ablation of the thyroid glands led to precocious development of the gonads (Thapliyal and Pandha[11]). In this bird, the thyroid activity is inversely related to the reproductive activity (Sinha[12]). Therefore, the rise in the