Brit. J. Pharmacol. (1952), 7, 438.

SOME PROPERTIES OF NISIN

BY

J. L. GOWANS, N. SMITH, AND H. W. FLOREY

From the Sir William Dunn School of Pathology, Oxford

(Received March 7, 1952)

Recently some attention has been devoted to the possibility that an antibiotic in particulate or colloidal form might possess advantages over small molecule water-soluble compounds in the treatment of tuberculosis (Markham and Florey, 1951; Markham, Heatley, Sanders, and Florey, 1951; Markham, Wells, Heatley, and Florey, 1951; Sanders, Florey, and Wells, 1951; Heatley, Gowans, Florey, and Sanders, 1952). It appeared that nisin, a relatively insoluble substance described by Mattick and Hirsch (1944), might have properties of value in this connection. Nisin is obtained from the culture fluid of a strain of streptococcus (group N). Mattick and Hirsch (1947) found that it inhibited strongly the growth of certain micro-organisms and that it was bactericidal at low concentrations.

The sample of nisin (Batch 263) used in most of the present experiments contained about 30,000 of the units of Mattick and Hirsch per mg. Assuming that the samples used by Mattick and Hirsch (1947) were of this order of purity, calculation from their figures shows that the growth of streptococci of groups A to M, except C, D, and L, was inhibited by concentrations of from 1 in 7,500,000 to 1 in 120,000,000 (0.25–4 units per ml.), and that of pneumococci Type 1 at 1 in 120,000,000 (0.25 unit per ml.). Some strains of neisseria, clostridia, corynebacteria, and actinomyces were inhibited at high dilution, while strains of staphylococcus and Type 2 pneumococcus were less sensitive.

From the point of view of the experiments being conducted in this laboratory the most important statement (Hirsch and Mattick, 1949) was that nisin was active against *Myco. tuberculosis*, a dilution of 1 in 50,000 to 300,000 (100 to 600 units per ml.) inhibiting growth in Long's medium, while as little as 1 to 3 units per ml. (1 in 10 to 30 millions) inhibited growth in Dubos and Davis's liquid medium. They also found that in certain circumstances it was possible to suppress strongly the development of tuberculosis in guinea-pigs and rabbits. Bavin, Beach, Falconer, and Friedmann (1952) have recently confirmed Hirsch and Mattick's finding that nisin is as active against *Myco. tuberculosis in vitro* as streptomycin. In their hands, however, nisin was therapeutically ineffective in mice infected with a virulent human strain of tubercle bacillus, and they attributed this to the difficulty of maintaining adequate blood levels of the drug. They also mentioned the possibility that their " nisin " might have been different from that used by Hirsch and Mattick. Nisin was reported in the above publication to be highly active against infections with *Str. pyogenes* and *Staph. aureus* in mice.

The following experiments were made possible by a gift of nisin from Dr. Mattick, and later by the presentation by Mr. B. D. Thornley, of Benger Laboratories, Ltd., of a number of samples made at different times on different media.

EXPERIMENTAL

Earlier workers had obtained indications that crude nisin contained more than one antibacterial substance. Accordingly, Berridge, Newton, and Abraham (1952) attempted to resolve a single batch of nisin (706P, prepared by Bengers Laboratories) into its components, by counter-current distribution between solvents. They showed that the crude nisin consisted of five distinct but related antibacterial polypeptides. The two main components (A and B) showed similar activity against *Str. agalactiae*. They accounted together for 81 per cent of the weight and 95 per cent of the total antibacterial activity of the crude material. The activity of the minor components C and D was about one-fifth that of A or B. The activity of the minor component E was not determined. These nisin polypeptides were similar in their amino acid composition and remarkable for their high content of sulphur. They all appeared to contain lanthionine and cystathionine or allocystathionine, substances not normally found in native proteins.

Some antibacterial tests have been made on separated components of crude nisin, but most of the work has been done on the crude substance. In the experiments to be described "nisin" refers to the crude substance (Batch 263) unless otherwise stated.

Physical and chemical properties of crude nisin

Batch 263 was stated to contain 30,000 units/mg. (about 75 per cent of the activity of Mattick and Hirsch's best preparation), and was a white powder readily soluble in water at *p*H 2, but precipitation occurred when the solution was neutralized. A solution in saline or in water at *p*H 7.4 appeared to be saturated with respect to the antibiotically active substances when about 0.1 mg./ml. was in solution.

Dialysis of a solution of nisin in saline at *p*H 7.4 in a cellophane sac against running tap water for 24 hours resulted in the loss of one-half to three-quarters of the activity. It appeared from this that the active substance might pass out of the blood stream with little difficulty.

Assay

The assay of nisin has presented difficulties and a number of methods have been proposed (Mattick and Hirsch, 1947; Hirsch, 1950; Friedmann and Epstein, 1951; Friedmann and Beach, 1951).

Throughout these experiments nisin was assayed by the cylinder-plate method using plates bulk-seeded with *Myco. phlei*. After the cylinders had been filled the plates were put into the refrigerator overnight to allow the solutions to diffuse into the agar. They were then incubated at 37° C. for 24 hours. Clear, sharp zones of inhibition were obtained. A solution of the hydrochloride in saline or in water, diluted to give a concentration of 0.1 mg./ml., had a *p*H of almost 7, and the assay of serial twofold dilutions of such a solution gave an approximately linear log. concentration zone diameter relation. Dilutions of a solution containing 0.1 mg./ml. in water provided the standards for the assay of unknown preparations.

To ascertain the fate of nisin in the body, tissues were extracted with 0.05 N-HCl, serially diluted in acid, neutralized, and spun; the concentration in the extract was calculated from those points that fell on the linear part of the assay curve. Serum and urine were assayed without any preliminary treatment.

For assay purposes it was arbitrarily decided that 1 mg. of the nisin powder should be regarded as containing 100 units; thus one of these units is equivalent to 300 of Mattick and Hirsch's units.

Antibacterial activity

Although nisin is very stable in acid solution, no satisfactory method was found for sterilizing nisin solutions, so they were prepared as far as possible with sterile ingredients and vessels. Boiling at *p*H 2 and filtration through sintered glass at *p*H 7.4 both resulted in loss of activity, especially against streptococci.

TABLE I

Crude nisin (Batch 706P) and its fractions A, B, C, D, and E tested against *Myco. tuberculosis* (H37Rv strain)

706P (crude nisin)		A		B		C		D		E	
Dilution	Days of incubation 4 8 12 16	Dilution	Days of incubation 4 8 12 16	Dilution	Days of incubation 4 8 12 16	Dilution	Days of incubation 4 8 12 16	Dilution	Days of incubation 4 8 12 16	Dilution	Days of incubation 4 8 12 16
1 : 100,000	— — — +	1 : 77,000	— — — —	1 : 84,700	— — — —	1 : 129,000	— — — —	1 : 20,000	— + + +	1 : 20,000	— + + +
200,000	— — + +	154,000	— — — —	169,400	— — — —	258,000	± + + +	40,000	+ + + +	40,000	— + + +
400,000	— + + +	308,000	± + + +	338,000	— + + +	516,000	+ + + +	80,000	+ + + +	80,000	+ + + +

— indicates no growth. ± indicates slight growth. + indicates growth equal to that in control cultures.

TABLE II

ANTIBACTERIAL ACTIVITY *in vitro* OF BATCH 706P AND ITS FRACTIONS

Highest dilution (1 in thousands or millions) giving complete inhibition at the time stated. All dilution series were in twofold steps. The Z strains of staphylococcus were supplied by Dr. Mattick

Nisin fraction	*Myco. tuberculosis*, H37Rv at 12 days	*Str. pyogenes* at 48 hours		*Staph. aureus* at 48 hours							
		Group A	Group C	Z80	Z83	Z88	Z89	Z92	167	CN 491	152*
A	154,000	9,856,000	616,000	9,856,000	4,928,000	308,000	9,856,000	19,712,000	1,232,000	2,464,000	616,000
B	169,400	10,841,600	677,600	5,420,800	2,710,400	169,400	5,420,800	10,841,600	1,355,200	2,710,400	338,800
C	<20,000	5,120,000		640,000		40,000				1,280,000	
D	129,000	16,512,000	1,032,000	4,128,000	4,128,000	258,000	8,256,000	16,512,000	1,032,000	2,064,000	1,032,000
E	<20,000	2,560,000		320,000		10,000				640,000	
Original powder 706P	100,000	12,800,000	800,000	6,400,000	6,400,000	200,000	12,800,000	25,600,000	1,600,000	1,600,000	400,000

* Penicillin-resistant strain

Crude nisin—Action on Myco. tuberculosis

The sensitivity of three virulent human strains and one avirulent human strain (H37Ra), and of eight bovine strains, was tested using crude nisin in a Dubos and Davis liquid medium. In no case was the extreme sensitivity of the bacilli, noted by Mattick and Hirsch in this medium, observed. The growth of one bovine strain was inhibited for 12 days at a dilution of 1 in 800,000, and the titres of the other strains ranged between 1 in 100,000 and 1 in 400,000.

Fractions of nisin

As it was possible that all the activity against *Myco. tuberculosis* might reside in one of the fractions isolated by Berridge, Newton, and Abraham, they were tested against *Myco. tuberculosis in vitro*, the results being shown in Table I. Fractions A and B were of about equal potency, so that it is clear that fractionation did not offer hope of securing a product much more active than the crude material. It was also clear that though crude nisin and some of its fractions inhibited the growth of tubercle bacilli *in vitro* they were, under conditions of test in this laboratory, considerably less active than streptomycin in similar circumstances.

Activity against organisms other than Myco. tuberculosis

Str. pyogenes.—Though different samples of crude nisin varied considerably in their activity, some of the best were extremely active against Group A strains. This great activity was shared by some of the fractions produced by Berridge, Newton, and Abraham (see Table II). In some experiments no growth of the CN 10 strain (of the Wellcome Laboratories) occurred after 48 hours' incubation in medium containing a concentration of 1 in 25,000,000.

Staph. aureus.—Some strains of this organism were very sensitive, though others were relatively insensitive. One strain was little affected by either nisin or penicillin, which confirmed the finding of Mattick (personal communication) with the same strain. Fractions A, B, and D were approximately as effective as the crude powder but, as with the streptococcus, fractions C and E were much less potent. These results are summarized in Table II.

Other samples of crude nisin showed considerable activity against staphylococcus (CN 491 strain of the Wellcome Laboratories), the concentrations giving complete inhibition at 48 hours varying from 1 in 0.8 million to 1 in 6.4 million.

Other bacteria.—Single strains of *Bact. coli*, *S. typhi*, *Sh. shigae*, *N. meningitidis*, and *N. catarrhalis* were found to be insensitive to nisin.

Effect of serum and tissues on the activity of nisin solutions in vitro

A nisin solution at *p*H 7 was diluted with an equal volume of horse serum and incubated at 37° C. for 24 hours. No loss of activity followed.

Incubation for two hours at 37° C. of a solution of nisin in water at *p*H 7 with slices of lung, liver, kidney, and spleen from a mouse did not result in any significant destruction of nisin.

The incubation of homogenized mouse lung, liver, kidney, and spleen with a nisin solution in water at *p*H 7 for two hours at 37° C. resulted in the loss of more than three-quarters of the activity of the nisin in all experiments.

Pharmacological investigations

Preparation of material for injection

Although nisin hydrochloride is quite soluble at *p*H 2 it is very insoluble at the hydrogen ion concentration of the blood. It was suspected that the toxic symptoms described by Hirsch and Mattick (1949) might have been due to rapid precipitation of nisin in the blood after the injection of an acid solution. That such precipitation did occur was made probable by the fact that a heavy precipitate occurred on injecting a hazy solution of nisin at *p*H 4 into serum in a test-tube. The symptoms and mode of death described by Hirsch and Mattick might well have been due to pulmonary emboli of nisin. Bavin *et al.* (1952) did not consider the possibility of causing embolism by the injection of their solutions at *p*H 4.

As a preliminary to tests of toxicity attempts were made to produce a suspension with fine particles at about *p*H 7.4. At this *p*H 5 mg./ml. of nisin could be dispersed in water by mechanical shaking to form a stable suspension whose particles showed Brownian movement. Unfortunately, a precipitate was immediately formed when such a suspension was added to normal saline or serum and no substance was found that protected the particles from flocculation. Material for intravenous injection into rabbits was made as follows.

About 50 mg. of nisin was dissolved in distilled water to give a solution containing 5 mg./ml.; 0.1 N-NaOH was added to this solution until a faint persistent haze had developed (about *p*H 4). Ten ml. of the hazy solution was drawn up into a syringe and then squirted rapidly through a long fine needle into 5 ml. of sterile rabbit serum in a McCartney bottle. The point of the needle was placed close to the bottom of the bottle in order that the particles of nisin might be broken up as they were precipitated. The resulting material was a milky suspension of nisin at about *p*H 7, containing particles up to about 60 μ in diameter. The *p*H was adjusted to 7.4 by adding 0.1 N-NaOH.

On injection into mice such suspensions readily killed by pulmonary embolism, but if the suspensions were first shaken for 10 to 15 minutes with ballotini beads on a Michel shaker mice often survived the injections without any signs of distress. This method of preparation was therefore adopted for intravenous injections. The suspensions for injection into mice contained 50 per cent rabbit serum; those injected into rabbits contained 33 per cent.

Toxicity after intravenous injections

To mice. Single injection.—The largest single dose given to a mouse weighing 20 g. was 8 mg. in 0.8 ml. The animal showed no immediate or delayed ill effects and no abnormalities were found when it was killed 4 weeks later. The maximum dose that could always be given to mice without ill effects was 1 mg. in about 0.3 ml.

Repeated injections.—Seven mice were given 8 daily injections of 1 mg. in a suspension containing 1 mg. in 0.3 ml. The animals were killed on the 9th day when in good condition.

The tissues of four of these animals were examined histologically. The livers had a few very small areas of necrosis with some cellular reaction. Small collections of cells were present in a few areas of the lung. In one animal there were areas in which the convoluted tubules of the kidneys were dilated and lined with flattened cells. In the others there were not such marked changes, though a few tubules appeared to be dilated. In some there were deposits of a homogeneous pink staining

material in some of the tubules. The glomeruli were unaffected. The spleens were apparently normal.

To rabbits. Repeated injections.—A rabbit weighing 3 kg. was given 870 mg. nisin in 18 daily injections of about 50 mg. The suspension contained 2.5 mg. nisin per ml. The rabbit remained well throughout the experiment and did not lose weight, but the veins of the ear progressively thrombosed. When it was killed 24 hours after the last injection the only macroscopic abnormality was a large spleen. Microscopically there was no striking change in the kidneys. A few tubules were somewhat dilated, but such changes were not so marked as in the kidneys of the mouse.

Three animals with experimental tuberculosis received about 1 g. during 3 weeks without any signs of distress.

Toxicity after subcutaneous injection

Four mice were given 5 mg. of nisin in 0.5 ml. of suspension subcutaneously. There was no necrosis, though slight initial reddening on the under surface of the skin was seen after 24 hours, when the first mouse was killed. At this time the mass of injected material had been invaded by polymorphs. Fourteen days later the area had become walled off by fibrous tissue which enclosed a fragmenting amorphous mass, presumably the remains of the nisin, studded with conglomerations of disintegrating cells. After a month no trace of the injected material could be found on naked-eye examination *post mortem.*

Toxicity after intrathecal injection

Suspensions and solutions were injected intrathecally into seven rabbits by the method described by Markham, Heatley, Sanders, and Florey (1951). Two rabbits, injected with 4.5 and 9 mg. of suspension respectively, did not recover consciousness after the anaesthesia and died after 32 and 33 hours. There was inflammation of the meninges with large numbers of granulocytes in the cerebrospinal fluid. The fluids gave zones of inhibition of 21 and 21.5 mm. respectively on plates seeded with *Myco. phlei.*

Four rabbits withstood without symptoms the injection of up to 0.1 mg. in solution in 1 ml. of saline at *p*H 7.4, but no nisin was detected in the cerebrospinal fluid at the end of 24 hours. The rabbits survived in good condition till they were killed after 2 months. One rabbit that received 0.12 mg. in solution died 3 hours after the injection, having had a series of fits. In this animal some difficulty was experienced in the preliminary withdrawal of cerebrospinal fluid, so that the fits and death may have been due to trauma, though this was not obvious at post-mortem inspection.

Toxicity to cells

Mackaness (1952) found that 50 μg. of crude nisin per ml. of culture fluid did not affect the motility of isolated macrophages.

Excretion

Urine from the bladders of five mice that had received 0.1 mg. (10 units) of nisin intravenously in 1 ml. of saline at *p*H 7 was collected at the end of 3 hours. Blood was collected from the cut inferior vena cava. For purposes of control, urine and

blood were collected in a similar way from five animals that had not received nisin. The urine secreted in 3 hours contained 1 unit/ml.; the serum at the end of 3 hours 0.08 units/ml.

In another experiment four mice were injected intravenously with nisin solution and two with nisin suspension. The mice were placed in pairs in glass cages, the floors of which were covered with four thicknesses of filter paper. After 24 hours the faeces were picked from the filter paper, which had been torn up by the mice. The paper was then extracted with 0.05 N-HCl. From the four mice that received 0.1 mg. (10 units) in 1 ml. of solution, 15 per cent of the injected material was recovered from the filter paper after 24 hours. In the same time only 3.6 per cent appeared to be excreted by the two mice that received 1.0 mg. suspended in 1.0 ml. (100 units).

These experiments made it clear that nisin is excreted by the kidneys, but neither rapidly nor completely.

Amount of nisin in serum

Mice were injected intravenously with 0.1 mg. (10 units) of nisin in 1 ml. of saline at *p*H 7. At various times after injection they were killed, the blood collected from a cut abdominal vein, and the serum assayed. Table III summarizes the results of two experiments. These experiments showed that some nisin remains in the blood for a matter of hours after injection.

TABLE III

THE AMOUNT OF NISIN IN THE SERA OF MICE AT VARIOUS TIMES AFTER THE INTRAVENOUS INJECTION OF 0.1 MG. (10 UNITS) OF NISIN

Each figure is derived from one mouse

Experiment 1		Experiment 2	
Time after injection	Units/ml. in serum	Time after injection	Units/ml. in serum
5 min.	0.96	6 min.	2.15
10 ,,	0.96	10 ,,	2.15
20 ,,	0.8	17 ,,	1.55
1 hr.	0.63	23 ,,	2.15
2 ,,	0.4	30 ,,	0.9
3 ,,	0.3	1 hr.	0.78

A trace of nisin was detected after 24 hours in the serum of the two mice that received 1 mg. (100 units) of nisin in suspension in the experiments described under " excretion," and in five out of seven mice that received 8 mg. of nisin suspension in 8 days (see next section).

Distribution in the tissues

Seven mice received eight daily intravenous injections of 1 mg. of nisin in 0.3 ml. of suspension. On the 9th day the animals were killed. Pieces of lung, liver, kidney, and spleen from three of the mice were extracted with 0.05 N-HCl and the extracts neutralized and assayed. Tissue extracts from untreated mice gave no zones of inhibition on the assay plates. The results are shown in Table IV.

TABLE IV

AMOUNT OF NISIN IN THE TISSUES OF MICE AFTER EIGHT DAILY INTRAVENOUS INJECTIONS OF 1 MG. FIGURES ARE UNITS PER G. OF WET TISSUE

Mouse	N 19	N 22	N 23
Lung	—	0.9	0
Liver	0.59	0	0.48
Spleen	2.2	1.1	1.0
Kidney	3.3	4.0	3.3

The large amounts in the kidney may perhaps be accounted for by embolism of the capsular capillaries, as was seen with micrococcin and micrococcin-Triton (Markham, Heatley, Sanders, and Florey, 1951; Heatley *et al.*, 1952). It is not surprising that the spleen should contain a high concentration as, no doubt, the phagocytic cells remove some of the material from the blood. The nisin in the liver may have been taken up by Kupffer cells.

Similar results were obtained with a rabbit that received 870 mg. in 18 daily injections. The assays were not as satisfactory as those from the mice, because extracts of tissues from an uninjected rabbit produce zones of inhibition. The spleen appeared to contain far more nisin than any other organ examined, and the amount in the kidneys was also large.

Animal protection experiments

Infection with Myco. tuberculosis.—Six albino rabbits weighing from 2 to 3 kg. were injected intravenously with 0.001 ml. of a 6-day culture of *Myco. tuberculosis* ("*Branch*" bovine strain) in Dubos and Davis's liquid medium. Beginning 5 days after infection, three of the rabbits were injected intravenously on 6 days each week with 50 mg. of nisin in 15 ml. of suspension. The other three rabbits were used as untreated control animals.

The daily intravenous injections caused no apparent distress, but there was progressive thrombosis of the ear veins. The details of the injections are shown in Table V. One control and one treated animal were killed at about 3, 5, and 7 weeks after infection.

TABLE V

DURATION OF EXPERIMENTS AND DETAILS OF NISIN INJECTIONS IN THE THERAPEUTIC EXPERIMENT AGAINST TUBERCULOUS INFECTION IN RABBITS

Number of infected rabbit	No. of days after infection rabbit killed	No. of injections of nisin	Total dose of nisin, g.
RN 13	22	15	0.75
RN 12	33	23	1.15
RN 14	47	23	1.15

Macroscopically the control and the treated animals showed extensive generalized tuberculosis, especially of the lungs. Lesions were present in the spleen and kidneys. No differences were noted between the two groups.

Microscopically the lungs of both treated and control groups showed confluent tubercles with caseation. In the spleens of the treated animals there was less extensive

disease and less caseation than in the controls, though numerous well-developed tubercles were present. There were no tubercles in the livers of the treated animals, but scattered giant cells were conspicuous. This contrasted with the livers of controls, where many microscopic tubercles without caseation were seen.

Tubercle bacilli were cultured from the lungs and spleens of all the control and treated animals.

Infection with Str. pyogenes.—Female mice weighing about 20 g. were infected intraperitoneally with 0.5 ml. of a diluted serum broth culture of a virulent *Str. pyogenes* (CN 10 strain). Treatment was started one hour after infection.

Effect of a single intravenous dose of nisin suspension.—Twenty-four mice were infected with a 1: 100 dilution of an 18-hour culture of *Str. pyogenes*. Ten mice were injected intravenously with 0.5 mg. nisin in a suspension containing 2.5 mg./ml. Fourteen mice served as untreated controls. All the control mice were dead 29 hours after infection. Seven out of ten of the treated mice survived and no abnormalities were found in them when they were killed 31 days after infection.

Effect of two intravenous doses of nisin suspension.—Twenty mice were infected with a 1: 100 dilution of a 17-hour culture of *Str. pyogenes*. Ten mice received an intravenous injection of nisin 1½ and 8 hours after infection, and ten mice served as control animals. Each dose contained 0.2 mg. of nisin in a suspension containing 2 mg./ml. All control animals were dead within 27 hours, while all treated animals survived. When they were killed 29 days after infection no abnormalities were found.

Infection with Staph. aureus. *Effect of local application.*—Five mice, infected intraperitoneally as described in the next section, were treated with a single intraperitoneal injection of 1 mg. nisin suspended in 1 ml. of water at *p*H 7.4. The mice survived for 7 weeks in apparently good condition, when they were killed. Three mice had large thick-walled abscesses in the peritoneal cavity, and two appeared to be normal.

Effect of a single intravenous dose.—Fifteen female mice weighing about 20 g. were infected intraperitoneally with 0.5 ml. of a 1: 1,000 dilution in 7 per cent mucin of a 24-hour broth culture of *Staph. aureus* (CN 491 strain). Six mice were treated with a single intravenous dose of 1 mg. nisin in 0.5 ml. of suspension. There was no significant prolongation of life, all the control mice dying within 24 hours and the last survivor of the treated group at 36 hours.

Effect of repeated intravenous injections.—After the effects of local application and of a single intravenous dose had been tested, six other crude samples of nisin became available. Of these Batch 754, produced in a peptone medium, proved to be the most active *in vitro* against the mouse virulent strain of staphylococcus, inhibiting growth for 48 hours at a dilution of 1 in 6.4 millions. This batch was accordingly used in a therapeutic experiment.

Forty mice were infected intraperitoneally with 0.5 ml. of a dilution in 7 per cent mucin of a 14-hour serum broth culture of *Staph. aureus*. Twenty mice received 1: 1,000 dilution of the culture; twenty received 1: 100,000 dilution. Ten mice receiving each dilution were treated with five intravenous injections of 0.5 mg. nisin in 0.2 ml. of suspension. Treatment was started one hour after infection and given twice a day for 2½ days. All the treated mice in the group infected with the smaller

inoculum survived and showed no abnormalities when they were killed 28 days later, whereas all the control mice were dead 26 hours after infection. In the other group all the control mice were dead at 12 hours, but none of the treated mice died from the infection during the period of observation; eight out of nine examined *post mortem* at 28 days had thick-walled staphylococcal abscesses in the abdominal cavity.

Discussion

The work just described was done to explore the possibility of using nisin for the treatment firstly of experimental tuberculosis, and secondly of tuberculosis in man. Many titrations with a number of samples of crude nisin against a number of strains of *Myco. tuberculosis* confirmed Mattick and Hirsch's statement that the material was active *in vitro* against this organism, but it was not possible to confirm that its potency was as great as or greater than that of streptomycin when tested in Dubos and Davis's liquid medium. The figures obtained with this medium were approximately the same as those obtained by Mattick and Hirsch, using Long's medium. Nor did the results *in vitro* accord with those of Bavin *et al.* (1951).

A number of fractions obtained from crude nisin by counter-current distribution (Berridge *et al.*, 1952) were not more active *in vitro* against *Myco. tuberculosis* than the crude substance. The trial of a good sample of nisin against tuberculous infection in the rabbit was discouraging. It was not possible to confirm the good results of Hirsch and Mattick, though the method of administration was somewhat different. The two experiments are set out for comparison in Table VI. Possibly

TABLE VI

DETAILS OF THE THERAPEUTIC EXPERIMENT AGAINST EXPERIMENTAL TUBERCULOUS INFECTION IN RABBITS COMPARED WITH THE EXPERIMENT DESCRIBED BY HIRSCH AND MATTICK (1949)

	Treament started: time after infection	Frequency of treatment	Daily dose, mg./kg.	Total dose, mg./kg.	Duration of treatment	Duration of experiment
Present experiments ..	5 days	daily	20	300–460	2–3 weeks	3–7 weeks
Hirsch and Mattick (1949)	12 hours	8-hourly	57	285	5 days	12 weeks

the present work was done with more virulent bacilli than those used by Mattick and Hirsch. Mackaness (1952) has shown *in vitro* that nisin is unable to inhibit the growth of the tubercle bacilli within macrophages even when 50 μg./ml. was present in the surrounding fluid.

It was not possible to do more experiments with rabbits, as the supplies of nisin were small, but such results as were obtained did not encourage the belief that other procedures would be more successful. In a private communication Dr. Mattick suggested that the nisin which was used in the present work might not be the same as that with which he and Hirsch originally worked. The findings here reported are in agreement with the negative results of Bavin *et al.* (1951).

The pharmacological results suggest that the toxicity following intravenous injection, reported by Hirsch and Mattick, was probably due to embolism of the

pulmonary vascular system. The fact that they found most nisin in the lung, unlike the results with a neutral suspension in the present observations, adds to this suggestion.

Nisin when administered in suspension or solution did not appear to be very toxic as judged by the appearance of the animals, though the finding of lesions in the kidneys of mice and of a rabbit after repeated doses suggests that nisin is not free from toxic action on the kidney associated with most of the polypeptide antibiotics. The focal lesions in the liver and lungs could be explained by the retention in these organs of substantial deposits of nisin around which an inflammatory reaction occurred, for when a suspension was injected subcutaneously at *p*H 7.4 it produced a smart polymorph reaction but it did not cause sloughing. When injected intrathecally as a suspension nisin caused sufficient damage to the cerebrospinal tissues to kill the animal. These observations are of some value in assessing the potentialities of nisin, for it has been proposed as a local treatment for bovine mastitis (Taylor, Hirsch, and Mattick, 1949). The substance undoubtedly is very active against some strains of streptococcus, and has rather less but still considerable activity against some strains of staphylococcus.

An antibiotic now has to have very remarkable properties to earn itself even a trial in human medicine. There are so many effective ways of dealing with infections by *Str. pyogenes* that the undoubted great protective effects of nisin in mice infected with this organism, in which the results of Mattick and Hirsch (1947) are confirmed, will probably never lead to a trial against natural infection in man.

Though its effects were less striking in staphylococcal infections in mice they were by no means unsatisfactory.

Infection by staphylococci resistant to penicillin and perhaps other antibiotics is a considerable clinical problem, and it is conceivable that the drug might be useful for local application in staphylococcal infections in man.

In view of its great potency against organisms commonly infecting wounds, it might be of value as a local prophylactic application.

SUMMARY

1. Nisin powerfully inhibits the growth *in vitro* of many strains of *Str. pyogenes* and *Staph. aureus*, but appears to be considerably less active against *Myco. tuberculosis* than previously reported. Fractions prepared from crude material had no greater activity against *Myco. tuberculosis* than the crude substance.

2. As nisin is only slightly soluble at the *p*H of blood its toxicity was tested by the intravenous injection of fine suspensions into mice. The largest single intravenous dose that caused no symptoms or abnormalities was 8 mg.

The intravenous injection of 1 mg. a day for 8 days caused no changes in the apparent well-being of mice, but histological examination disclosed small areas of necrosis in the liver and slight changes in the tubules of the kidneys.

Intravenous injection of 870 mg. into a rabbit weighing 3 kg. over a period of 18 days produced no striking effects.

Nisin in suspension injected subcutaneously became encapsulated.

Intrathecal injection of 4.5 and 9 mg. of suspension in two rabbits produced coma.

The substance, which was partly excreted by the kidneys, could be detected in the blood for some hours after injection.

3. Under certain conditions it is effective against experimental infection of mice with *Str. pyogenes* and *Staph. aureus.*

4. No effect was demonstrated against infection with a bovine strain of *Myco. tuberculosis* in rabbits.

We are indebted to Mr. J. Kent, Mr. D. W. Jerrome, Mr. I. Chinn, and Miss M. Lancaster for technical assistance and to Dr. M. A. Jennings and Miss W. M. Poynton for help in the preparation of the manuscript.

References

Bavin, E. M., Beach, A. S., Falconer, R., and Friedmann, R. (1952). *Lancet, i,* 127.
Berridge, N. J., Newton, G. G. F., and Abraham, E. P. (1952). *Biochem. J.* (in press).
Friedmann, R., and Beach, S. A. (1951). *J. gen. Microbiol.*, **5**, Proc. v.
Friedmann, R., and Epstein, C. (1951). *J. gen. Microbiol.*, **5**, 830.
Heatley, N. G., Gowans, J. L., Florey, H. W., and Sanders, A. G. (1952). *Brit. J. exp. Path.*, **33**, 105.
Hirsch, A. (1950). *J. gen. Microbiol.*, **4**, 70.
Hirsch, A., and Mattick, A. T. R. (1949). *Lancet, ii,* 190.
Mackaness, G. B. (1952). *J. Path. Bact.*, **64**, 429.
Markham, N. P., and Florey, H. W. (1951). *Brit. J. exp. Path.*, **32**, 25.
Markham, N. P., Heatley, N. G., Sanders, A. G., and Florey, H. W. (1951). *Brit. J. exp. Path.*, **32**, 136.
Markham, N. P., Wells, A. Q., Heatley, N. G., and Florey, H. W. (1951). *Brit. J. exp. Path.*, **32**, 353.
Mattick, A. T. R., and Hirsch, A. (1944). *Nature*, **154**, 551.
Mattick, A. T. R., and Hirsch, A. (1947). *Lancet, ii,* 5.
Sanders, A. G., Florey, H. W., and Wells, A. Q. (1951). *Brit. J. exp. Path.*, **32**, 452.
Taylor, J. I., Hirsch, A., and Mattick, A. T. R. (1949). *Vet. Rec.*, **61**, 197.