INTRACUTANEOUS INJECTION OF ACIDOMYCIN (SATANI'S METHOD) IN THE TREATMENT OF LEPROSY

PRELIMINARY REPORT

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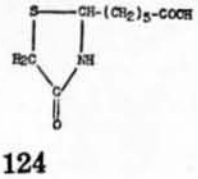
Acidomycin, an antibiotic active against *Mycobacterium tuberculosis in vitro,* was obtained from a culture of *Streptomyces acidomyceticus* and its variant first isolated and named by Koichi Ogata of the Takeda Ferment Institute. The microbiological study of this streptomyces was first reported at a meeting in Hiroshima in 1951, and then at another held in Tokyo in 1952 (8, 9, 10). Since the summer of 1950, studies have been carried out on the purification of this new antibiotic and the determination of its chemical structure. Before this work was completed, however, Ogata and his associates learned that this same antibiotic had been isolated independently by groups at Chas. Pfizer and Company (11, 12), the Abbott Laboratories (1, 3), the Lederle Laboratories (13), and at the National Institute of Health in Tokyo (15), and that it had been synthesized by the first two of these groups.

ISOLATION AND PROPERTIES OF THE ANTIBIOTIC

Isolation and structure.—The culture-broth containing the acidomycin is filtered from the mycelium at pH 4.0 (5). The acidified solution (pH 2.0) is extracted with an equal volume of butyl acetate. On concentrating the butyl acetate solution to one-eighth the original volume, acidomycin begins to crystallize. Recrystallization from methanol gives long colorless needles m. p. 137.5-138.5 (uncorr.), $\alpha_D^{15} = -46.2°$ (c = 0.073 g. in 25 cc. of methanol). Data of elementary analysis are in agreement with $C_9H_{15}O_3NS$. The antibiotic is readily soluble in methanol, ethanol, buanol, chloroform, acetone, ethyl acetate, butyl acetate, benzyl-alcohol, and hot water; it is less soluble in ether, petroleum ether, benzene and cold water. The ultraviolet spectrum of acidomycin has no characteristic absorption in the region of 200 to 360 mμ. Solutions of acidomycin in water and organic solvents exhibit a blue fluorescence on exposure to ultraviolet light.

After the molecular formula was obtained in the Takeda Laboratory the report of Solomons *et al.* (12) was received, and it was found that acidomycin closely resembles Solomons' 2-(5-carboxypentyl)-4-thiazolidone.



The compound was therefore synthesized according to the Solomon's method, and it was established by a mixed melting point determination that acidomycin is identical with his compound.

Pharmaceutical action.—Acidomycin exhibits high specific activity *in vitro* against the human and avian types of tubercle bacilli (6). It is active against the human type H_2 strain at a concentration of 0.3 γ per cc. against a streptomycin-resistant strain of human type (Suita strain) at a concentration of 1.22 γ per cc. by the dilution method, and against the Frankfurt human strain at a concentration of 0.5 γ per cc. by the slide-culture method of assay.

Toxicity.—The toxicity (LD_{50}) of acidomycin injected intravenously in mice is 35 mgm. per 10 gm., and when injected subcutaneously 200 mgm. per 10 gm. The toxicity is therefore very low compared with that of streptomycin.

Biological experiment.—Mitsuo Hori *et al.* (2) of the Tuberculosis Department of the Institute of Microbial Diseases, Osaka University, reported inhibitory action on the growth of tubercle bacilli by acidomycin in submerged culture. In their experiments, Actinomyces 2222, I and II (both identical with acidomycin), showed so powerful an inhibitory effect *in vitro* on the growth of human and avian types of tubercle bacilli that complete checking was observed at 3.2 millions dilution, and a very strong action even at 12.8 millions dilution.

On the contrary, experiments with this antibiotic carried out in both Japan and the United States showed no antituberculosis activity *in vivo*. Negative results have been obtained both in animal experiments and in the treatment of the human disease. A slight effect was seen in only one case which was treated by injecting acidomycin directly into a tuberculous cavity of the lung, at the 3rd Internal Medicine Clinic of the Osaka University Hospital. The treatment of tuberculous skin eruptions with acidomycin ointment had no curative effect.

The lack of an antituberculosis action of this antibiotic in the animal and human experiments is said to be due to the existence of biotin in the body fluid (4, 14). Biotin, even in small quantities, has a very strong antagonistic effect on acidomycin administered by intravenous or subcutaneous injection.

Also, no curative action was seen in another experiment conducted in the Dermatological Institute of Osaka University (7), in which acidomycin was employed in the treatment of murine leprosy. In short, the results with acidomycin have been disappointing as regards an antituberculosis action in biological experiments.

PRESENT EXPERIMENTS

One of us (the senior author) tested the activity of acidomycin in cases of leprosy by means of intracutaneous injection and obtained very interesting results. Since the body fluid in the superficial layer of the skin is very small in quantity, the concentration of biotin there seems to be nearly zero. Therefore, acidomycin applied into the cutis should encounter very little antagonistic influence. (The intensity and activity of other medicines and reagents would also be changed if they were applied intracutaneously.)

Acidomycin was injected intracutaneously directly into the leprous lesion. In the treatment, 0.2-0.3 cc. of solutions containing 10-40 mgm. per cc. of acidomycin were injected into different parts of the macules, 2 or 3 times a week. The injection was made cautiously, with a very thin needle, in passing through the epithelial layer into the cutis propria

or subcutaneous tissue. After a few injections the area surrounding the injected sites gradually became pale in color. The elevation due to the leprous infiltration also flattened down to the level of the healthy skin, and finally the lesion disappeared entirely after 2 to 6 months of injections.

The following are brief reports of fifteen cases treated by intracutaneous injections of acidomycin, arranged in type groups. Six were tuberculoid, 6 were lepromatous, and 3 were neural.

CASE 1. K. T., female, 30 years old, tuberculoid. Onset 16 months before. Previous treatment had been Promin injections for 2 months, and concomitant INH and streptomycin (SM) for 10 months. From May 1953, 1.0 cc. acidomycin (10 mgm. per cc.) was injected intracutaneously into macules on the upper arm and the forehead, 3 times a week. By the time a total of 1,040 mgm. had been given over a period of 7 months the macules had faded, leaving areas of leucoderma. Injections were temporarily stopped in March 1954, after about 11 months of treatment (1,780 mgm.). There was marked improvement, and the elevated purplish plaques on the forehead had flattened and become light red in color, with the capillaries visible. Bacteriological examinations were not made. *Verdict*: Very effective.

CASE 2. K. N., female, 30 years old, tuberculoid. Eight years duration. INH and SM previously for 7 months. Intracutaneous injection in the margin of a macule on the back was started in August 1953. A total of 420 mgm. was administered in 4 months, with some improvement, but when the total had reached 1,300 mgm. (8 months) there was an "acute infiltration" reaction and the macules on the back, chest and face became markedly elevated. In May 1954, after 9 months of treatment (1,460 mgm.), there being no improvement treatment was stopped. No bacteriological examinations. *Verdict*: Worsening effect.

CASE 3. T. K., male, 19 years old, tuberculoid. Onset 6 months before. Promin for 3 months. October 1953, started injections in a macule on the right upper leg. Five months later (1,130 mgm.), there was marked improvement, with fading and flattening; sensations of pain and touch had recovered. Treatment was stopped after 8 months (1,990 mgm.), the sense of touch greatly restored. A macule on the opposite upper leg was also improved, with fading of color and flattening. Bacteriologically negative. *Verdict*: Very effective.

CASE. 4. T. H., male, 13 years old, tuberculoid. Onset 1 year before. Promin injections for 5 months. A macule on right cheek was treated from October 1953, and in 2 months (360 mgm.) it had improved, the margin becoming ill-defined; also, macules on the left forearm and hand showed improvement. After 6 months (1,010 mgm.) the nodular enlargement of the right auricularis magnus had markedly decreased in size. After 8 months (1,690 mgm.) only a trace of the cheek lesion was discernable, and the auricularis could barely be palpated. No bacteriological examinations. Treatment is being continued. *Verdict*: Very effective.

CASE 5. S. B., female, 64 years old, tuberculoid. One year since onset. Previously, Promin for 2 months. A macular lesion on the left buttock treated from October 1953. When 390 mgm. had been injected (2 months) the margin became ill-defined, and macules located elsewhere showed improvement. Eight months later (2,140 mgm.), the treated lesion had almost disappeared, sense of touch recovered. Bacteriological examinations negative. *Verdict*: Very effective.

CASE 6. K. M., male, 64 years old, tuberculoid. Onset 46 years before. No previous treatment. Injections of a macule on the back started October 1953. Two months later (360 mgm.) the surface became scaly, with decrease in elevation. After 8 months (2,050 mgm.) the color had faded and it had become still flatter. Negative for bacilli. *Verdict*: Somewhat effective.

CASE 7. M. E., female, 20 years old, lepromatous. Onset 18 months previously. Chaulmoogra had been given for a year, and Promin plus P_{54} for 10 months. Beginning October 1953, intracutaneous injections of 1.0 cc. of the (1%) acidomycin solution into the margin of an infiltrated, bacteriologically positive area. After 4 months (910 mgm.) the color of the infiltration had faded somewhat and the elevation decreased, but bacilli were still present. Treatment was temporarily stopped after 6 months (1,390 mgm.). There was still some discoloration of the area, but it was almost level with the healthy skin. Still positive for bacilli. *Verdict*: Moderately effective.

CASE 8. T. N., female, 30 years old, lepromatous. Duration 9 months. Promin for 6 months. Injections were made into an infiltration on the calf of the left leg. After 2 months (240 mgm.) there was flattening and some fading. At 8 months (1,940 mgm.) the color had almost completely faded, and the area was almost level with the healthy parts. Bacilli not found. Treatment is being continued. *Verdict*: Very effective.

CASE 9. K. N., male, 30 years old, lepromatous. Seven years duration. Previously chaulmoogra, together with Promin, for 1 year, and P_{54} therapy for 10 months. Acidomycin treatment of a bacillus-positive infiltration on the back started in May 1953. After 6 months (890 mgm.) there was some improvement. One year later (2,350 mgm.) the treated lesion, and also infiltrations elsewhere, showed marked improvement, with fading in color and flattening. Bacilli, however, were still found. Treatment is being continued. *Verdict*: Very effective.

CASE 10. T. Y., female, 31 years old, lepromatous. Onset 13 years before. No previous treatment. An infiltrative lesion in the forehead, positive for bacilli, was treated from October 1953, with no change after 2 months (340 mgm.), but worsening at 3 months (430 mgm.), so treatment was stopped. *Verdict*: Worsening effect.

CASE 11. A. F., male, 23 years old, lepromatous. Duration 8 years. Prior treatment, Promin 1 year, and SM plus INH 10 months. Injections of an infiltration on the back started May 1953. After 7 months (1,017 mgm.), there was improvement not only in that lesion but in others all over the body, with flattening and fading. Treatment was stopped after 1 year (2,190 mgm.). The lesions had flattened almost level with the healthy skin, and the color had faded even more markedly. Bacilli, however, were still found. *Verdict*: Somewhat effective.

CASE 12. Y. N., male, 51 years old, lepromatous. Onset 9 years before. Promin for 2 years. Treatment of an infiltrative lesion on the abdomen started October 1953. Some improvement after 2 months (360 mgm.), becoming marked by 7 months (1,500 mgm.). Not only the lesion treated but also other lesions showed decolorization and flattening. Bacilli still positive. *Verdict*: Somewhat effective.

CASE 13. Y. M., male, 14 years old, neural (leucoderma). Duration four months. Promin for 2 months. Injections in the margin of a leucodermic lesion, left buttock, begun in October 1953. After 8 months (1,850 mgm.), there was some coloration of the area, but treatment was stopped. *Verdict*: Ineffective.

CASE 14. S. Y., male, 30 years old, neural (leucoderma). Eleven years since onset. Previously, Promin for 3 months, chaulmoogra for 1 year, and P_{54} for 8 months. In October 1953 treatment of a leucodermic lesion on the chest was begun. It was stopped after 7 months (1,360 mgm.), there having been no change in the lesion. No bacteriological examination. *Verdict*: Ineffective.

CASE 15. K. K., male, 51 years old, neural. Duration 11 years. Promin for 6 months. An area of leucoderma on the back injected from October 1953. No change was observed after 8 months (1,880 mgm.), but treatment is being continued. Lesion negative for bacilli. *Verdict:* Ineffective.

DISCUSSION

Appreciable results have been obtained by the injection of acidomycin intracutaneously in leprosy cases, the injections being given directly into the cutis of the lesions treated. The dosage varied, but usually it was 0.2-0.3 cc. of a solution containing 40 mgm. per cc. (i.e., 4%). Of the 15 cases here reported, 6 were tuberculoid, 6 lepromatous, and 3 neural. Among the tuberculoid cases, the treatment was found to be very effective in 4 and moderately so in 1; in 1 case there was worsening by reaction. In the lepromatous cases the treatment showed less marked results: in only 2 was it recorded as very effective, the benefit in 3 others being only moderate; again, 1 case showed worsening. In all 3 neural cases it was quite ineffective.

In a majority of cases, after a few injections the macule faded in color where the antibiotic had been injected, and gradually the fading spread to the surrounding area, so that the entire macule might disappear without trace within a few months. The plateau-like elevation of the macule also became flat and level with the skin surface, paralleled by the fading in color. The sensitivity of the faded macule and repigmented leucoderma recovered gradually, also in parallel with the change of skin color. Lepra bacilli could not be found in smears from the faded macules in 2 cases, and they showed an obvious decrease in 1 other case.

It was very interesting to note disappearance of macules at a distance from the injected sites. This occurred in 3 tuberculoid cases (Nos. 3, 4 and 5), and in 3 lepromatous cases (Nos. 9, 11 and 12), with less general effect in the latter than in the former, since in only 1 was the treatment recorded as "very effective" whereas it was so recorded in all of the 3 tuberculoids. In one of them even a thickened auricularis magnus subsided greatly.

From the facts observed, intracutaneous injection of acidomycin may be said to have a very desirable action in the treatment of leprosy. A number of experiments have been carried out in the Aisei-en and Keifu-en leprosaria and elsewhere to investigate the results of our experiments.

We are planning to test the influence of acidomycin upon leprosy lesions when it is injected into healthy parts of the skin of patients, to ascertain the curative effect on noninjected and distant lesions.

Moreover, we have been testing the effect of acidomycin applied intracutaneously in the treatment of tuberculosis in the same manner as in leprosy cases, and have been obtaining very interesting results which will be reported later. A study of the effect of intracutaneous injection of the antibiotic is being carried out with tuberculous rabbits and guinea-pigs infected with the bovine and human types.

SUMMARY

1. Acidomycin, a new antibiotic prepared in the Takata Laboratory, has been employed in the treatment of leprosy.

2. In this experiment the antibiotic was injected superficially into the leprous skin lesions, because when injected subcutaneously or intravenously acidomycin has shown no therapeutic effect in either tuberculosis or leprosy, although it is very active against the tubercle bacillus *in vitro*.

3. With intracutaneous application, on the contrary, there has been demonstrated a very interesting action. Where it was effective there was fading or disappearance of the macules and improvement or recovery of sensitivity. In certain cases there was decrease or disappearance of bacilli in the faded macules.

4. In several cases, lesions at a distance from those that were injected also showed improvement. In one of them a nodular auricularis magnus nerve became much reduced.

RESÚMEN

Un nuevo antibiótico, la ácidomicina, ha sido ensayado en el tratamiento de las lesiones leprosas. Este antibiótico fué descubierto independientemente por dos instituciones del Japón y tres de las mayores casas farmacéuticas de los Estados Unidos. Es muy activo contra el bacilo tuberculoso *in vitro*, pero debido al antagonismo de la biotina, se ha mostrado absolutamente ineficaz contra la tuberculosis humana o experimental y contra la lepra cuando se administra subcutánea o intravenosamente.

En el experimento aquí descrito, se inyectó en lesiones cutáneas de lepra muy superficialmente, en cuyo sitio hay aparentemente muy poca cantidad del antagonista. Por este método, resultó ser beneficioso en la mayor parte de los 15 casos tratados. Estos comprendían 6 tuberculoideos, 6 lepromatosos y 6 neurales. Cuando la droga surtía efecto, había descoloramiento o desaparición de las manchas y mejoramiento o recuperación de la sensibilidad, y en ciertos casos una mejoria bacteriológica correspondiente. En varios casos, también mostraron mejoria lesiones alejadas de las inyectadas. En uno de ellos, hubo mucha reducción de la nodulación de un nervio auricular.

Esta investigación continúa y se va ampliando.

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