Acute Peri-Articular Inflammation Induced in Rats by Oral 6-Sulfanilylindazole. (29687)

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Experimental inflammation of the joints of ankles and feet in rats ('polyarthritis') can be induced by local injections of irritants(1), or by remote injections of systemically active agents such as micro-organisms(1-5), hormones(1), or immunological agents(1,6-9). We have not found in the literature any report of an orally active agent. Inflammation of the periarticular connective tissue of ankles and feet in rats following oral administration of 6-sulfanilylindazole was originally observed by T. J. Becker and J. O. Hoppe at this Institute during a routine toxicity test (unpublished) in 1946. This report describes the pathology and pathogenesis of this phenomenon.

Materials and methods. Male albino Sprague-Dawley rats weighing 100, 300, or 500 g were used. A total of 445 rats were medicated with 6-sulfanilylindazole (designated as SAI hereafter) by gavage 6 times weekly for various periods. The doses were 62.5, 125, 250, or 500 mg/kg given as a suspension in 1% gum tragacanth. Twelve rats received SAI subcutaneously at 125 mg/kg, and 61 control rats received the gum tragacanth vehicle by gavage.

Inflammation was recorded daily by measuring the width of the ankles (distance from lateral to median malleoli). The rats were sacrificed and autopsied at various intervals from the 5th to the 36th days of medication. Feet and portions of the major organs were processed for microscopic examination.

To determine whether the SAI induced inflammation was species-specific, SAI was also administered by gavage to mature male rhesus monkeys (*Macaca mulatta*) and to mature male albino Swiss mice. The doses of SAI were 62.5, 125, and 250 mg/kg; each dose was administered to 3 monkeys 25 times in 29 days, and to 10 mice 18 times in 21 days. Control groups of 4 monkeys and 10 mice received only the vehicle, 1% gum tragacanth. All animals were observed daily for changes in appearance and behavior. Blood urea nitrogen determinations were done on monkeys weekly as an indicator of kidney toxicity due to the sulfonamide. All mice that died during the experiment were autopsied.

Subcutaneous connective tissue of feet and cardiac blood from 3 rats with inflamed feet and 3 control rats were cultured for bacteria and PPLO (pleuropneumonia-like organisms). Blood-agar plates, beef heart broth, and thioglycollate broth were used for bacteriological cultures, and pancreatic digest medium(10) containing 25% sterile normal horse serum was used for PPLO cultures. PPLO growths were identified when stained according to Dienes' method(11).

Results. Inflammation of the feet in rats medicated with SAI usually appeared between the 5th and 10th days of medication, but, infrequently, the onset was as early as the 2nd or as late as the 18th day of medication. The inflammation occurred almost exclusively in the hind feet. Front feet were affected very rarely and no signs of inflammation were observed in the tail or spine or in the digits if the total foot was not inflamed.

The first signs of inflammation were slight swelling and reddening of the ankle, which increased in severity until the whole foot and the lower part of the shank were involved. At the peak of inflammation, usually 2 to 3 days after the onset, the skin of the affected area was bluish red, severely distended, and serous fluid oozed through the skin. The width of the ankle was increased from a normal width of approximately 7 to 7.4 mm to approximately 11 mm. The peak of inflammation (Fig. 1) lasted for 2 to 3 days, then the inflammation slowly subsided although daily medications with SAI were continued. Fourteen to 21 days after onset, the erythema

disappeared completely, but a slight indurated swelling of the ankle remained and the flexibility of the ankle joint was severely reduced.

Not all cases of inflammation became intense. At times, the initial slight swelling and redness did not increase; in such cases the slight erythema disappeared at 14 to 21 days, but the slight swelling remained as induration.

Microscopic studies of the inflamed feet at the acute stage revealed severely edematous subcutaneous tissue infiltrated by polymorphonuclear leucocytes. Most of these leucocytes were located adjacent to the bone (Fig. 2). During the 3 or 4 days after the acute stage, most of the polymorphonuclear leucocytes were replaced by mononucleated leucocytes. Some of these mononucleated cells had the characteristics of osteoblasts and the interstitial substance between them was dense. Six to eight days after the acute stage, the previously edematous areas consisted of masses of collagenous fibers and fibroblasts, and the intercellular substance adjacent to the distal end of tibia was partially ossified. At this time also the articular synovial membranes were invaded by polyblasts. Approximately 30 days after the onset of inflammation (Fig. 3), newly-formed bone at the distal ends of tibias was microscopically indistinguishable from the old bone. Cavities of the new bone contained osteoclasts and bone marrow-like cells. The affected synovial membranes were hypertrophied and they filled the synovial sacs. However, the articular surfaces or the spaces between them were not affected.

The incidence of peri-articular inflammation in the orally medicated rats was directly related both to the dose of SAI and to the age of the rats (Table I).

SAI was well tolerated at the lower dose levels, but doses of 250 to 500 mg/kg resulted in suppression of growth rate in the younger (100 g) rats and in loss of body weight and occasional deaths in the older (500 g) rats.

At autopsy, fibrous adhesions surrounding the spleen were observed in approximately one-half of the affected rats. No other sig-

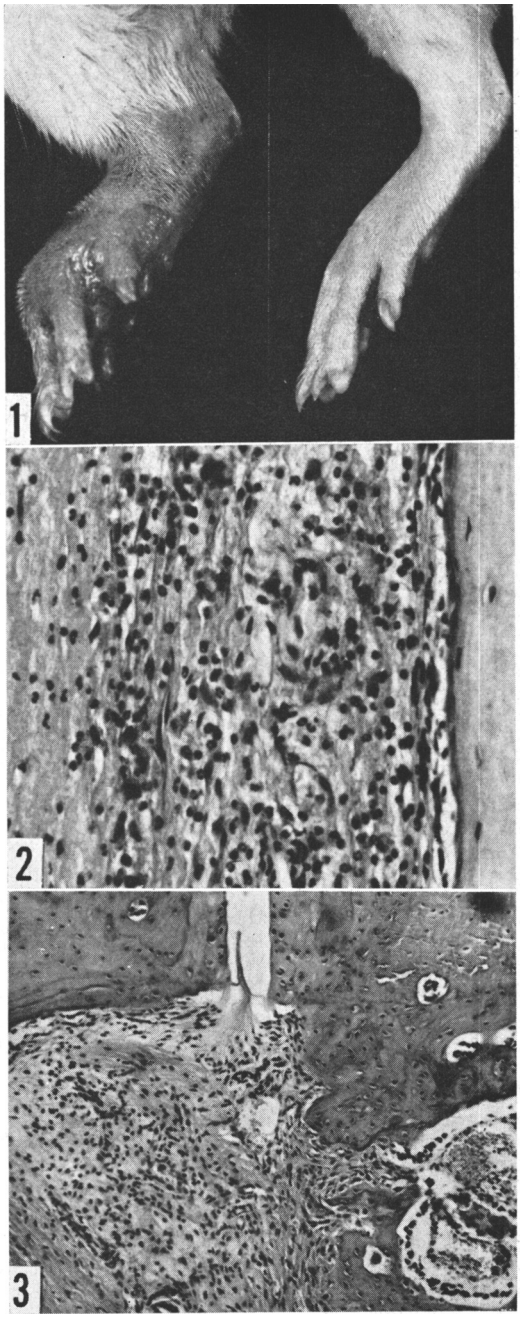

FIG. 1. Left: gross appearance of acutely inflamed foot. Right: normal foot.

FIG. 2. Acutely inflamed periosteal area of distal end of a tibia. 288 ×.

FIG. 3. A tarsal joint 30 days after acute stage of inflammation. Completely ossified new bone at lower right with bone marrow-like cavities. Fibrous hypertrophy of synovial membranes and complete obliteration of synovial sac. 113 ×.

TABLE I. Incidence of Peri-Articular Inflammation in Rats Medicated Orally with 6-Sulfanilylindazole.

Approx rat body wt (g)	Daily oral dose of SAI in mg/kg for 18-22 days					
	0	62.5	125	250	500	750
100	0/30	0/5	0/5	2/60	30/136	8/8*
300	0/6	0/6	3/6	6/6	[27/42]†	
500	0/25	10/13	117/123	18/18	25/25	

* 3/8 died during 14-day medication period (on 9th and 12th days). Data from the original observations by Becker and Hoppe (unpublished).
† Medicated for 5 days only.

nificant gross changes were observed in any of the major organs.

In monkeys and mice, daily oral medication with SAI did not result in signs of inflammation of ankles or feet: nephrotoxicity (high blood urea nitrogen values) was noted in the monkeys, and 18 of the 20 mice medicated with SAI at high doses died of bronchopneumonia.

The bacteriological examination of subcutaneous connective tissue and cardiac blood did not yield bacterial growth. However, the cultures of the inflamed tissues and the heart blood of all 3 medicated rats were positive for PPLO, whereas PPLO were not recovered from the cultures of normal control rats. The PPLO colonies were identified in the third to seventh sub-cultures; culture media controls were negative.

Discussion. The inflammation induced by oral administration of SAI was characterized by productive inflammatory changes of the subcutaneous connective tissue primarily around the ankles of the hind limbs. This inflammation eventually resulted in fibrosis of the connective tissue of the feet and formation of new bone at the distal ends of tibias. The foci of inflammation were not migratory and the articular surfaces were not affected within the 36-day observation period. Because the inflammation affected the subcutaneous and periosteal connective tissue primarily, the term "arthritis" was deemed to be inappropriate for the SAI-induced disease.

In considering the etiology of the SAI-induced inflammation, the following observations are relevant:

1. The incidence of inflammation was high in old rats and low in young rats. 2. Reports in the literature indicate that older rats harbor PPLO more frequently than younger rats (12), and in rats some strains of PPLO may have an affinity for the interstitial connective tissue(13). 3. PPLO were recovered from the inflamed tissues and cardiac blood of the affected rats whereas bacteria were not. PPLO were not recovered from the control rats. 4. The appearance of the SAI-induced inflammation was similar to that of rats infected intravenously with PPLO as described by Ward and Jones(2). However, suppuration in the intra-articular spaces and spinal involvement were not observed in the rats medicated with SAI.

These observations suggest that oral administration of relatively large doses of SAI and appearance of PPLO infection were correlative. Although no evidence was obtained that crystals or high local concentration of SAI could induce local inflammation, acute systemic PPLO infection in young rats is known to be capable of inducing an "arthritis"(2,3) which is similar to the inflammatory changes induced by orally administered SAI. In our studies, the frequency distribution of inflammation in relation to the dose of SAI and the age of rats suggests that the inflammation was dependent upon the presence of a latent PPLO infection which was activated by SAI. If the drug-induced response is dependent upon the incidence of an age-related latent PPLO infection, the suggested etiology is compatible with the more uniform susceptibility of young rats, earlier onset, and possibly greater severity of the disease caused by intravenous injection of PPLO(2).

The observed inflammation of lungs of mice medicated with SAI may be related to the prevalence of a catarrhal strain of PPLO in the respiratory tract of mice(14).

Summary. Inflammation of the peri-articu-

lar connective tissue of ankles and feet in rats has been regularly induced by oral administration of 6-sulfanilylindazole (SAI). The incidence of this inflammation probably has been caused by drug-induced provocation of latent PPLO infection. The mechanism of this effect of SAI is not known.

The authors are indebted to Dr. H. P. Drobeck for helpful contributions in preparation of the manuscript. The authors are also indebted to Miss Sophie Hansen for her careful studies on PPLO and to Mr. Joseph V. Sansone for skillful technical assistance.

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Received September 2, 1964. P.S.E.B.M., 1964, v117.

Effect of Hydrocortisone on Alkaline Phosphatase in Cultured Cancer Cells.* (29688)

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Cox and MacLeod(1) have shown that prednisolone increased the level of alkaline phosphatase in HeLa cells. Melnykovych(2) studied the effects of a large number of steroids on HeLa cells and observed only glucocorticoids increased the alkaline phosphatase activity. Maio and DeCarli(3) reported that alkaline phosphatase activity is increased by prednisolone or hydrocortisone in isolated clones of cell strain EUE which normally exhibit a low enzyme activity. Clones which possessed high activity were not stimulated by prednisolone or hydrocortisone. The work reported here involved a study of some effects of prolonged hydrocortisone exposure on J111, H.Ep2 as well as HeLa alkaline phosphatase activity.

Materials and methods. Normal HeLa S_3B, J111 and H.Ep2 cells and those grown in the presence of 25 μg/ml hydrocortisone acetate for 6-8 weeks were grown in 50 ml Erlenmeyer flasks for six days at 37°C in 42.5% H-16 and 15% Saline F medium(4) supplemented with 15% calf serum. The cultures were started from an inoculum of 10^5 cells per ml per flask. After 3 days the old medium was replaced with fresh medium and incubated for another 3 days. The cells exposed to hydrocortisone were grown for an additional 3 days.

Determination of alkaline phosphatase activity was conducted according to the method of Melnykovych(2). At the end of the incubation period the medium was withdrawn by suction and the glass adherent cells were washed 3 times with 0.5 ml of balanced salt solution. To each flask was added 2.0 ml of 0.5 M glycine buffer pH 9.1, 0.5 ml of 0.05 M $MgCl_2$ and 0.4 ml distilled water. The flasks were shaken on an Aminco-Dubinoff shaker at 37°C for 30 minutes and 0.5 ml of substrate containing 1 mg of p-nitrophenylphosphate was added to each flask.

* Supported by Contract with A.E.C. #AT(11-1)-394.