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LESIONS IN RATS GIVEN SULFAGUANIDINE IN PURIFIED DIETS [1]

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In a preliminary paper (*1*) the occurrence of calcified vessels in young rats receiving sulfaguanidine was reported. In subsequent reports (*2*, *3*) other lesions were recorded or briefly described. The rats were fed a purified diet deficient in B complex and containing 1 percent sulfaguanidine. In addition they received supplements of riboflavin, thiamin, pyridoxine, pantothenic acid, nicotinic acid, and choline. Some received further supplements of impure biotin concentrates.

It is the purpose of this report to describe in detail the vascular, cardiac, muscular (skeletal), and hepatic lesions which have been observed in rats on the above regimen.

Arteries.—The most frequent early alteration found histologically in arteries was a thin layer of basophilic homogeneous material coating short segments of the elastic fibers of the media or the internal elastic lamina. Less frequently observed were short segments of vessel wall in which muscle fibers were absent and elastic fibers less distinctly outlined. Such segments appeared relatively homogeneous and somewhat refractile. In some instances the basophilic material was seen only on the intimal side of the internal elastic lamina. Occasionally it was present in sufficient amount to cause localized elevation of the intima. When the basophilic layers coating the elastic fibers were thick, smooth muscle cells were quite indistinct marginally and their cytoplasm much less oxyphilic than normal. In many vessels the entire intima and media were basophilic and usually showed none of the normal histologic structures; occasionally a short segment of an elastic fiber could be recognized. The location and degree of involvement was quite variable. In a cross section of a given vessel the

[1] From the Divisions of Pathology and Chemotherapy, National Institute of Health.

involvement may occur only in one small area. It may involve multiple thin and short segments at various depths in the wall, or the entire thickness of the wall may be basophilic in single or multiple segments. Finally, the entire wall may be converted (at a given level) into a relatively rigid tube which does not contract after death (fig. 1C). In the latter condition the inner surface of the wall was smooth and the vessel appeared dilated. When there was total involvement, elastic fibers usually could not be demonstrated. The thin lamina or short segments of involvement usually were sharply delineated and in many instances marginated by relatively normal wall (figs. 1D and E). Sometimes the muscle fibers adjacent to such an area were swollen, showed decreased oxyphilia of their cytoplasm, and occasionally spindle shaped nuclei were increased in number.

Although fairly homogeneous in the early lesions, the basophilic wall of the extensively involved vessels showed some variation in density or depth of staining and in some instances was distinctly granular. As previously reported, this material forms a brownish red lake with alizarin red S, is brown to black when stained by the von Kossa method, and is brownish gray after staining by the Van Gieson technique. These typical color reactions, together with evident sclerosis of the vascular walls, identifies the basophilic material as calcium.

A variation of the pathologic picture described above was seen in a smaller number of vessels. In these, the vessel wall was refractile or "glassy" in appearance and was amphophilic and much less deeply stained than those described above. The wall was quite brittle, and in routine sections (not decalcified) it was usually broken into multiple variably sized plates often with serrated margins. This was in contrast to the deeply basophilic wall which, although fracturing to some degree, usually retained its normal outline. Occasionally elastic fibers, particularly the internal elastic lamina, could be distinguished in the hyalinized and calcified wall.

Necrosis of vessel walls was observed in a few animals but only in a very small percentage of the total number of altered vessels. Necrosis occurred more often in coronary vessels than in any other location. It was found only in vessels which also showed calcification. In these it was restricted to small areas usually located between calcified segments. In such areas the wall was thickened, oxyphilic, and granular, but showed no cellular exudation.

The adventitia of calcified vessels usually showed no increase in cellularity or of connective tissue fibers. Rarely such an increase was present but of slight degree. Also there was no evidence that any vessel had fractured or ruptured previous to the death of the animal; perivascular hemorrhage was not encountered. Two coronary arteries contained recent thrombi, and two others showed incomplete

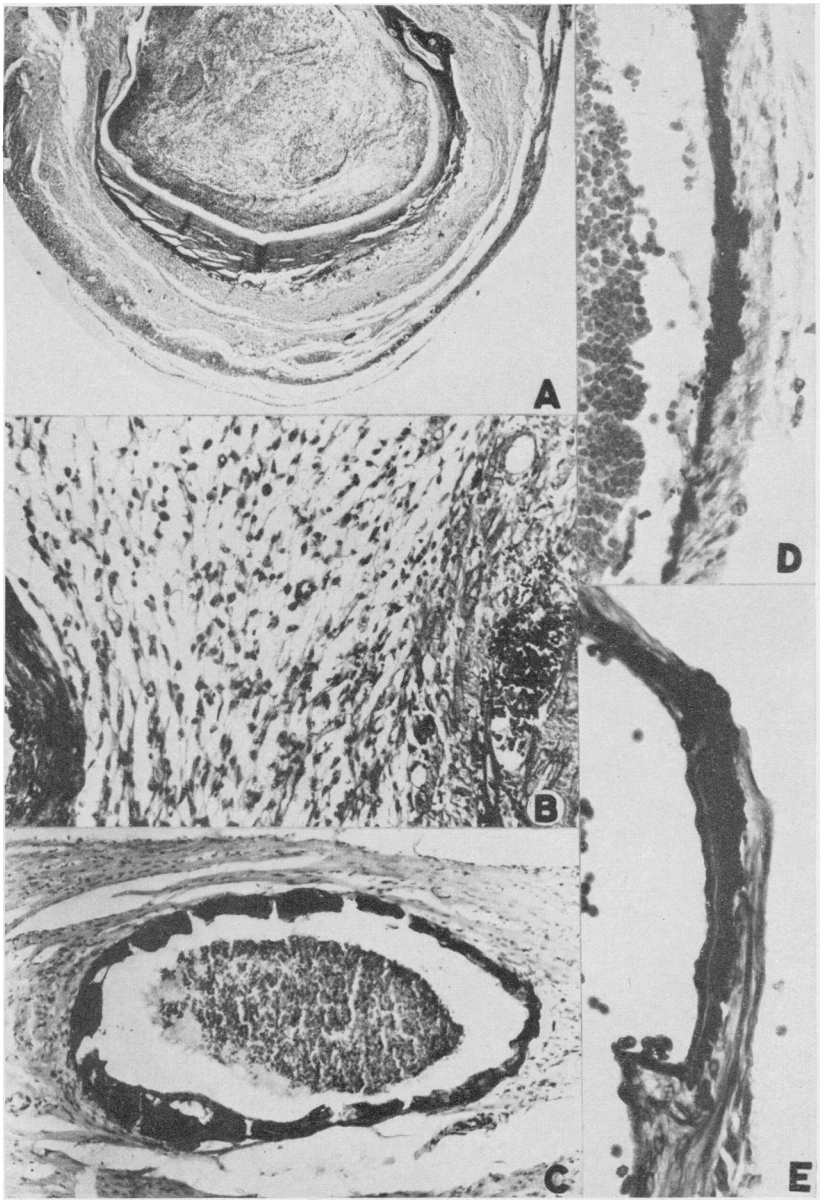

FIGURE 1.—(A) Apical portion of dilated left ventricle filled with blood and showing a laminated mural thrombus. The myocardium is subtotally replaced by loose scar tissue. Note the thin subepicardial layer of muscle fibers. ×15. (B) Shows the entire thickness of a left ventricle wall at apex. Subepicardial muscle fibers at left and a small portion of a thrombus at lower right. Loose connective tissue forms most of the wall in this area. ×167. (C) Completely calcified coronary artery. ×150. (D and E) Partially calcified renal arteries. Note the noncalcified internal elastic lamina in E. ×370.

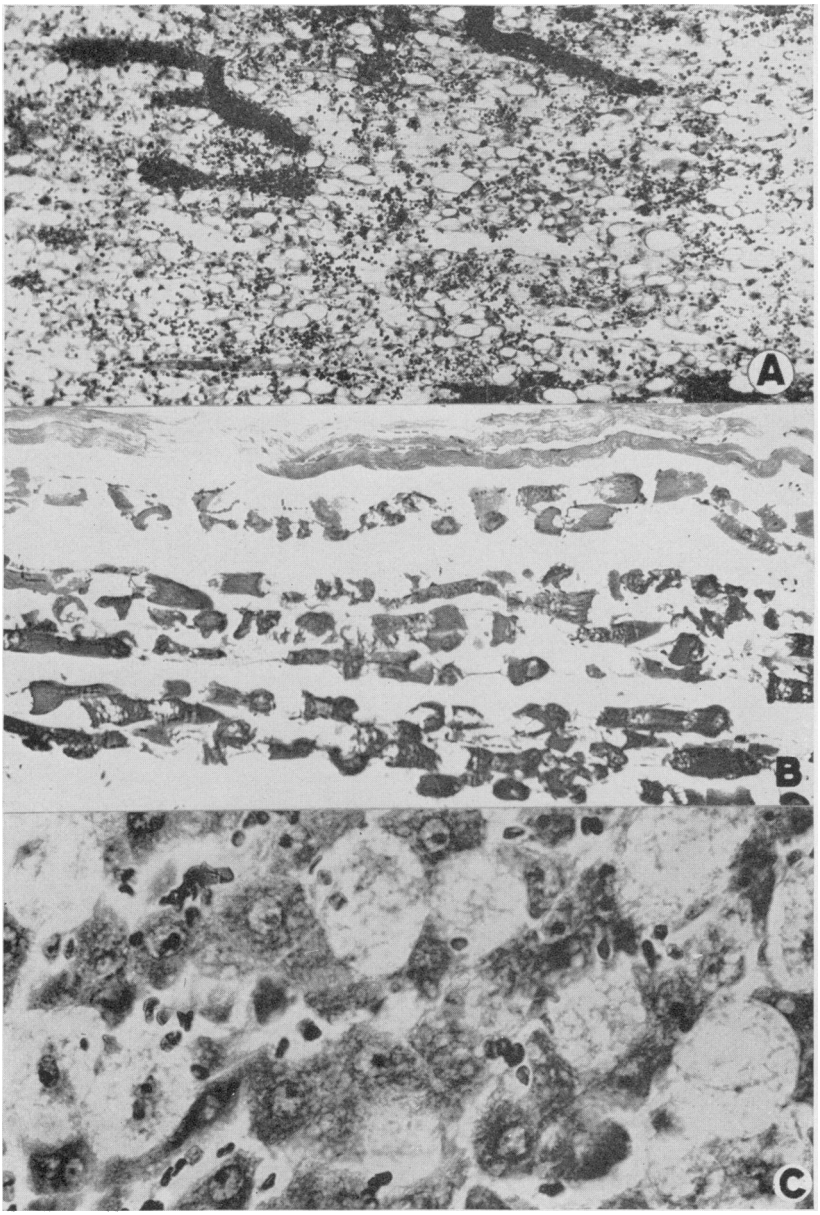

FIGURE 2.—(A) Marrow from mid-portion of tibial shaft showing aplasia of granulocytes. Most of the few remaining cells belong to the erythropoietic series. ×143. (B) Hyalinized, necrotic, and fragmented skeletal muscle fibers. Contrast with the relatively normal fibers in upper part of photomicrograph. ×143. (C) Liver, showing greatly enlarged hydropic cells. At lower left two involved cells show recognizable nuclei of liver cell type. ×940.

obstruction of their lumens by extremely loose connective tissue. These will be considered further in the discussion.

Although in the majority of cases the involved vessel could be recognized as an artery, there was an occasional one in which such recognition was uncertain. On the other hand, focal calcification was observed rarely in the larger pulmonary veins. In the rat these veins possess cardiac type of muscle in their mediae.

Calcified arteries have been found in the lungs, heart, kidney, pancreas, stomach, intestines, mesentery, thyroid, mediastinum, and thymus. The above organs and tissues are listed in order of frequency in which vessel calcification was observed in the series of animals examined to date. These organs (excepting the thymus) and also the liver, aorta, skeletal muscle, esophagus, tibia, vertebra, and bone marrow were routinely examined histologically. Testis, thymus, and skin and subcutaneous tissue were examined with less regularity.

In the lungs only the larger pulmonary vessels at or near the hilus were involved. Arteries at the hilus of the kidney were calcified more often than those within the kidney. Such involved vessels were rarely seen in the cortex. In other organs and tissues (excepting the heart which will be discussed later), the involved vessels were of variable size and showed no consistent pattern distribution.

Heart.—In general the lesions of the coronary arteries were more severe than in the arteries of other organs. Involvement of coronary arteries occurred with about equal frequency in the right and left ventricles and in the interventricular septum. Such vessels were not found at or quite near the apex, and only rarely was a calcified artery seen in the lower half of the ventricles or septum. In a few hearts the sections passed through the orifice of a coronary artery. In such cases where the coronary was calcified in its first portion, the involvement stopped sharply at the junction of coronary and aorta. The aorta, usually the ascending portion, was regularly examined, but never showed pathologic alteration.

Lesions in the ventricular myocardium were present in 31 cases. The frequency of cardiac lesions cannot be determined satisfactorily since the time of their appearance is somewhat later than other lesions, and many animals died or were killed before the cardiac disease had had a chance to develop. In three hearts the involvement consisted only of a few minute to small foci of interstitial fibroblast proliferation or fibrosis. In the others, there were either foci of coagulation necrosis of muscle fibers, or recent scarring, or both. The recent (loose) scars were seen more often at and in the vicinity of the apex although both ventricles and septum were similarly altered in about one half of the cases. The degree of alteration decreased toward the base and only in a few cases was there myocardial damage present in the upper third of the heart. The scars were formed generally of a loose fibrillary

network with a moderate number of capillaries. In small foci there appeared to be little or no increase in connective tissue, the scar being a result of muscle fiber removal with collapse and condensation of the pre-existing interstitial tissue. In some areas the fibers were numerous, compactly disposed, and stained as collagen in the Van Gieson technique, whereas in others the fibers were much more distinct when stained by Masson's trichrome stain. The cellularity of such scars was variable in degree, usually sparse and never dense. Most cells were of fixed connective tissue type, although a few macrophages and rare lymphocytes and neutrophils were seen in a few cases. Capillaries and venules appeared increased in number and occasionally were distended with blood.

As seen in section the scars at the apex were often in the form of a band extending for a short distance into both ventricular walls toward the base of the heart. In most instances the band-like area occurred in the middle of the myocardium leaving a relatively unaltered internal and external muscle layer. In severely damaged hearts the scarring often approached or reached the endocardium at some point but usually left an intact but thin and interstitially fibrosed subepicardial muscle layer. In these cases the scar usually extended into the septum and one or both ventricles about half way to the base of the heart. This band of loose connective tissue as seen in section was occasionally interrupted by intervening patches of relatively normal myocardium. Similar scarring was observed occasionally in papillary muscles and less frequently around calcified arteries in the upper half of the ventricles. A few isolated muscle fibers were occasionally present in the loose scar tissue.

Muscle necrosis was of coagulation type. In most instances the fibers still retained their form and rarely was there interstitial connective tissue proliferation in such areas. The necrotic foci generally were of small to medium size and were most commonly observed adjacent to or near the scars. In some hearts multiple isolated foci of necrosis were seen, and in one, necrosis without scarring was present. Rarely, a few calcified fibers were noted. Mural thrombi, in the left ventricle, left auricle, or both, were present in about one-third of the hearts showing myocardial damage. Some showed organization, focal calcification, or both. As would be expected, thrombi were more frequent in the hearts with extensive pathologic alteration. In these cases the left ventricle wall at and near the apex was markedly thinned and the ventricle dilated and filled with blood. In three instances bulging of the wall at the apex was observed grossly suggesting beginning or actual cardiac aneurysm.

Skeletal muscle.—Hyaline necrosis or calcification of skeletal muscle was a common finding. The muscles examined included those from the leg, thigh, esophagus, and trunk. Often there was moderate

variation in degree of involvement in different muscle bundles as judged from single sections of the muscles examined. In some cases only an occasional altered muscle fiber was found; in others, single or multiple small groups of such fibers were present, and, in a few animals, all fibers of one or more muscle bundles were necrotic. Occasionally, degenerating fibers were present in the leg or thigh muscles and not in the paravertebral or esophageal muscles, or vice versa. Muscle fibers of the esophagus were necrotic less frequently than those from other locations.

Some muscle fibers showed indistinctness of or lack of cross striations, some were hyalinized but intact, others showed complete hyaline necrosis with fragmentation and retraction of the segments. In some of the hyalinized necrotic fibers there were poorly marginated vacuoles or areas of decreased density. A few muscle fibers showed distinct granular degeneration and decreased oxyphilia. In many animals an occasional calcified fiber was present and in a few cases there was complete calcification of most fibers in one or more muscle bundles. Such extensive calcification was recognized at autopsy as white gritty streaks or bands. In esophagi showing necrosis of muscle, a few calcified fibers were frequently observed, and in two animals all muscle fibers in the level examined were calcified.

A frequent associated finding was the focal proliferation of compactly grouped and often coherent large mononuclear cells, usually surrounding necrotic but clearly recognizable muscle fibers. In some cellular foci only oxyphilic debris was present and in a few the necrotic fibers had been completely removed. The cells surrounding the degenerating fibers, although occasionally discrete, frequently had indistinct peripheral margins and often they were coherent. In many instances the fibers were enclosed by large cytoplasmic masses having many deeply stained nuclei. The cytoplasm of these cells was slightly oxyphilic or amphophilic and their nuclei oval or round. The difficulty of differentiating myoblasts from histiocytes in this location is recognized but it is believed that most of the cells were of the latter type. Regenerating muscle fibers were rarely observed.

Liver.—Slight to moderate increase in size of liver cells and their nuclei, with nuclear hyperchromasia, was frequently observed. Also mitoses occurred with more than average frequency. A less frequent but more striking finding was marked edematous swelling of scattered single or grouped liver cells. These cells were very large, often three to four times average size, and in routine paraffin sections had clear or markedly vacuolated cytoplasm. Such cells occasionally showed a central, small, often pyknotic nucleus, held in position by a few radiating threads of cytoplasm. In frozen sections the nucleus was usually in a central or slightly eccentric position and the cytoplasm markedly vacuolated, but much less often collapsed than in paraffin

sections. Staining with Sudan IV and Best's carmine showed that neither fat nor glycogen was responsible for the liver cell enlargement and cytoplasmic vacuolation. When such cells were infrequent they were scattered irregularly; when numerous, they occurred in variably sized groups or bands which were fairly sharply delineated from adjacent uninvolved liver. In a few instances the involvement was clearly centrolobular, in one it was distinctly midzonal, but in most cases there was no consistent distribution pattern of the involved cells. Of the cases showing the hydropic liver cells, the involvement was slight in 40 percent, moderate in 40 percent, and marked in the remainder. In the latter group, one-half to three-fourths of all liver cells were edematous. In addition, a few livers showed small focal or larger centrolobular areas of necrosis. Since such necrosis is occasionally seen in control rats, the significance of this finding cannot for the present be evaluated.

Bone marrow.—The changes which occur in bone marrow were described in a report (*3*) dealing with the agranulocytosis which develops with regularity in these animals. Briefly, the bone marrow alteration consists of from slight to subtotal depletion of cells of the granulocytic series. Mature cells first disappear but when the aplasia is marked, only a few very immature granulocytes remain. In this stage there is often moderate to marked congestion.

Hemorrhage.—The occasional occurrence of hemorrhage into various organs and tissues warrants little description for the present. In most instances the hemorrhage was of recent origin and usually there was very little associated change in the involved tissues. The most frequent sites of the hemorrhages were the skin and subcutaneous tissue and the testis.

DISCUSSION

The sequence of events which ends in calcification of vessels does not appear to follow the same pattern regularly. Frank necrosis of vessel walls was observed in a minority of animals and usually only in small segments or foci in vessels which were also partially calcified. In a few vessels a little granular calcific material was observed in a short oxyphilic and necrotic segment, suggesting that granular necrosis occasionally preceded calcification. The deposition of calcium in layers along elastic fibers was seen in many vessels. The progression of this process with eventual fusion of such layers appears a more satisfactory explanation of the pathogenesis of most vascular lesions. In some vessels hyalinized segments were observed. The diffuse deposition of calcium in such an altered wall would be expected to result in a brittle and somewhat refractile structure. This type of alteration was present in one or more vessels of many animals. In rare instances material staining like calcium was observed in the cytoplasm of viable cells.

The lesions in the myocardium appear to be, first, patchy necrosis of muscle fibers at or near the apex, followed by the formation of a loose fibrillary "scar," and finally by fibrosis. As the process advances at the apex, occasionally with aneurysmal bulging of the left ventricle, the areas of involvement extend toward the base of the heart.

The mode of action of sulfaguanidine in producing the lesions described has been discussed in previous reports (*1*, *2*, *3*). The lowering of the intestinal synthesis of essential growth factors, direct toxic action, and the inhibition of certain enzyme systems were considered. The cardiac necrosis may well be produced in the same manner as other lesions. However, it appears that the sclerotic coronary arteries play a part in the progression of the cardiac disease if not in its initiation. Supporting this belief is the fact that the main coronary arteries and their larger branches in the upper two-thirds of the ventricles are the ones involved whereas the myocardium farthest away from the blood supply is usually first and most severely damaged; also muscle fibers subjacent to endocardium and epicardium are least frequently involved.

If the diseased coronary arteries are accepted as contributing to the myocardial damage, it must be assumed that this is effected in most cases by a disturbance of circulation other than blockage. Of the large number of calcified arteries studied to date, there were only two which were thrombosed. One was small, noncalcified, and located in a recent myocardial scar. The other vessel, which had a calcified wall, contained a fibrin thrombus which partly blocked the lumen. This heart had areas of myocardial damage much older than the thrombus. Also, two calcified coronary arteries showed subtotal obstruction of their lumen by sparsely cellular, very loose connective tissue. It was impossible in these cases to determine whether this condition was a result of thrombosis or intimal proliferation. It is recognized that sclerosed coronary arteries with constricted lumina cause nutritional disturbances in the myocardium resulting in the disappearance of muscle fibers and scar formation. It seems reasonable to assume that circulatory disturbances and nutritional deficiency can also occur as a result of rigid calcified coronary arteries without constricted lumina. These arteries in the areas of involvement have lost their distensibility and contractility which may slow the rate of blood flow. Also the loss of contractility may reduce the time during which normal intravascular pressure is maintained. However, this may be slight since the coronary system is so close to the highly elastic aorta.

The necrosis and calcification of skeletal muscle, often with histiocyte proliferation, are similar to that described by Olcott (*4*) and Pappenheimer (*5*) as occurring in the young of female rats fed a diet

deficient in vitamin E. However, the interstitial edema and fibrin and cellular exudate, noted by these and other authors, have not been observed in the muscles of our rats fed sulfaguanidine. It is realized that the diet used in this experiment contains marginal or possibly deficient amounts of vitamin E, yet a hyalinized or necrotic muscle fiber is seen only occasionally in control rats. Evidence has been advanced to indicate that biotin and vitamin K are synthesized by the bacteria of the gastrointestinal tract of the rat (*6*, *7*). It is known that sulfaguanidine inhibits the growth of bacteria in this location (*8*). If vitamin E were also synthesized by intestinal bacteria of the rat, it would seem plausible that the ingestion of this drug in a marginal diet might lead to a frank vitamin E deficiency.

SUMMARY

Rats fed a purified diet deficient in B complex and containing 1 percent sulfaguanidine regularly develop lesions of blood vessels, voluntary muscles, and bone marrow, and less often lesions of the heart and liver, and hemorrhages into various organs and subcutaneous tissues. All of the animals received supplements of riboflavin, thiamin, pyridoxine, pantothenic acid, choline, and nicotinic acid, and some received additional supplements of impure biotin concentrates. Arterial lesions consist of focal to extensive calcification, less often hyalinization or necrosis. These lesions were found in the lungs, heart, kidney, pancreas, thyroid, thymus, stomach, intestines, and mediastinum. Hyalin necrosis of skeletal muscle with or without calcification was found in all locations thus far examined. The muscles were those from leg, thigh, trunk, and esophagus. Lesions of the heart consist of necrosis of muscle, followed by the formation of loose, sparsely cellular scars. Often there is marked thinning of the left ventricular wall with dilatation, particularly at or near the apex. Many animals show marked hydropic swelling of isolated or grouped liver cells. In some instances more than half of all liver cells are involved. Slight to marked bone marrow aplasia (granulocytes) was observed in a majority of the animals.

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THE TRANSMISSION OF *PLASMODIUM LOPHURAE*, AN AVIAN MALARIA PARASITE, BY *ANOPHELES QUADRIMACULATUS*

By Herbert S. Hurlbut and Redginal Hewitt*

The experimental infection of *Anopheles quadrimaculatus* with *Plasmodium lophurae* has been reported by Coggeshall (*1*, *2*) and Hurlbut and Hewitt (*3*). The mosquito transmission of this parasite by *A. quadrimaculatus* has not been reported previously so far as the writers are aware. Laird (*4*) has recently reported successful transmission with *Aëdes albopictus*.

Specimens of *A. quadrimaculatus* were fed on infected ducks having a gametocyte count of approximately 1 per 100 erythrocytes and kept at 74° to 80° F. during the development of the exogenous stages. Stomach dissections indicated that about 20 percent became oöcyst positive. The mosquitoes were allowed to bite 4-week-old ducks after a lapse of 17 to 27 days. Parasites were first observed in the blood of three out of four ducks so treated, 13, 13, and 23 days after the infective feedings. One of the birds had been splenectomized previous to infection. The infection in this specimen was more intense and of longer duration than in the other two ducks. In all cases the number of parasites which appeared in the blood following the bites of infected mosquitoes was very low, never exceeding more than one per thin-field. The data are presented in table 1.

Table 1.—*The mosquito transmission of* P. lophurae

Bird Number	Number of mosquitoes biting [1]	Development in the mosquito—days from infective feeding to transmission (74°–80° F.)	In the duck	
			Prepatent period (days)	Patent period (days)
HW5	19	23	23	7
HW60	10	17	13	5
HC103 [2]	10	[3] 19–27	13	12
HW72	6	23	No infection	

[1] Dissections had shown about 20 percent oöcyst positive.
[2] Splenectomized prior to infection.
[3] Bird HC103 was infected by mosquitoes of several lots in which the time from infective feeding to transmission varied from 19 to 27 days.

Observations may be summarized as follows: The exogenous stages of this parasite were completed in about 17 days in *A. quadri-*

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maculatus at 74° to 80° F. The rate of oöcyst development suggests that this is close to the minimum time at this temperature. The prepatent period in 4-week-old ducks was 13 to 23 days. The level of parasitemia was low and the patent period of relatively short duration. Splenectomy prior to infection in one duck resulted in an infection of somewhat greater intensity than in nonsplenectomized ducks.

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SULFANILAMIDE IN THE TREATMENT OF LEPROSY [1]

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The introduction of sulfanilamide as a potent chemotherapeutic agent for combating certain types of invasive bacterial diseases and the striking results obtained from its use have led to a widespread trial of the drug. Bacteriological experiments by Long and Bliss (*2*) tend to prove that sulfanilamide does not in itself kill the microorganisms but exerts a bacteriostatic effect which aids the normal defenses of the body in overcoming infection. The administration of sulfanilamide and related compounds has been associated with definite although not necessarily unavoidable or serious hazards. In a recent article, Long and his associates (*3*) analyzed the toxic manifestations which occurred during the course of treatment with this drug in one thousand cases at the Johns Hopkins Hospital. The most common toxic effects were headache, dizziness, nausea, vomiting, cyanosis, drug fever, and drug rashes. The most serious toxic manifestations, however, were those associated with the blood or hemopoietic system and the liver (hepatitis).

Impressed by the action of sulfanilamide on other diseases, the writers decided upon its experimental administration to combat secondary infection in leprosy. They also wished to see if it would have an influence on the disease itself if given over a sufficient period of time and in sufficient dosage to produce an effective blood level.

First course.—At the beginning of the experiment nine patients, eight males and one female, were chosen from a group of volunteers. Eight of these cases were lepromatous and one was neural, although they all showed some neural manifestations.

Certain preliminary laboratory procedures were carried out in all cases. As a measure of kidney function, the urea clearance test was

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made, in addition to a complete urinalysis and estimations of the nonprotein nitrogen and the urea nitrogen of the blood. A complete blood picture was made, this including the erythrocyte count, hemoglobin estimation (Sahli's method), total and differential leukocyte counts, and the sedimentation test (Cutler's method). Because of the toxic effect of the drug on the hemopoietic system, the determination of the blood elements was repeated every second day during the course of the treatment, and in some instances every day. Urinalyses were also made frequently. Patients with markedly impaired renal function were not chosen, because sulfanilamide is largely excreted by the kidneys and its accumulation in the body would give rise to toxic manifestations.

Long and Bliss consider 4 to 8 mg. percent of sulfanilamide an effective blood level in patients with mild or moderately severe tissue infection, and 10 to 15 mg. percent in patients who are severely ill. The plan adopted at the outset was to give 15 grains (about 1 gm.) of sulfanilamide and 15 grains of sodium bicarbonate at 4-hour intervals, four times daily for 6 days, then the same doses twice daily for 6 weeks. With this dosage the free sulfanilamide concentration of the blood ranged between 5.5 and 16.6 mg. percent, with an average of 9.0 mg.

Only two of these patients completed the 6-week course of treatment without toxic manifestations. The other seven were all hospitalized for fever ranging from 38° to 41° C. and other toxic disturbances. In these cases the treatment had to be discontinued between the sixth and the twenty-first days because of the following complications: drug fever, 4; neuritis, 1; drug dermatitis, 1; and hepatitis, 1. The amount of sulfanilamide taken by these patients varied from 315 to 1,650 grains (20 to 105 gm.).

One of these patients was critically ill. Jaundice was noted on the sixth day, and it was felt that a toxic hepatitis was developing. The liver and spleen were palpably enlarged. The icteric index was 50 units. The free sulfanilamide blood concentration was 16.6 mg. percent, and that of nonprotein nitrogen was 40 mg. percent. The drug was discontinued. Three days later the patient became delirious and the surface of the body was cold and clammy. Dextrose, 5 percent in physiologic saline, was administered by vein and subcutaneously, and the patient improved. Two weeks later the icteric index was 20 units. Within 1 month the weight increased 23 pounds and was maintained.

Second course.—After a rest period of 2 months, a second series of treatments was given to 6 of the above group and also to an additional 11 patients, 11 males and 6 females. This made a total of 20 patients who received sulfanilamide therapy, 14 taking one course and 6 taking two courses. Those taking the second course included 1 neural case and 16 active lepromatous ones which varied in degree of the disease from early to advanced.

The same laboratory analyses as before were carried out on this group before initiating treatment, but the subsequent examinations were made twice a week instead of every second day, unless it became necessary to do so more often. Because of the severe toxic reactions experienced in the first group, it was thought advisable to decrease the dose of the drug for the second course. The plan adopted was to try to obtain a sulfanilamide blood level between 4 and 8 mg. percent. To maintain this level, 15 grains of sulfanilamide and 15 grains of sodium bicarbonate were given at 4-hour intervals, four times daily for 2 days, after which the dose was lowered to 10 grains of each drug, three times a day for 12 weeks. The free sulfanilamide concentration of the blood of these patients was found to range between 3.2 and 9.0 mg. percent, with an average of 5.0 mg.

In this series the total number of days of treatment ranged from 3 to 92, and the total dosage varied from 75 to 2,880 grains (5 to 190 gm.). Six of the patients completed the entire course. It was necessary to hospitalize 12 of the group because of toxic reactions or fever, and the medication was discontinued in 11 of them. In spite of a continuous fever for more than a week, during which time the evening dose of the drug was omitted, the other case was able to complete the course of 88 days of treatment without further ill effects.

Altogether the drug had to be discontinued on account of high continuous fever in six cases. In one case progressive anemia was the reason for interrupting the treatment; in another it was persistent neuritis, and in still another, recurrent hepatitis.

One case suffered from cerebral depression followed by semicoma after the sixteenth day of treatment. She became critcally ill, with a temperature of 40° C. and a leukocyte count of 50,200 per cmm., with 94 percent neutrophiles (37 staff cells and 57 segmented cells). The blood nonprotein nitrogen was 50 mg. percent; the urine was negative for albumin, sugar, blood, and casts. After discontinuing the drug, 1,000 cc. of 5 percent dextrose in phvsiologic saline was given intravenously, two such doses being administered 6 hours apart. Two days later the patient was markedly improved.

Another case developed a rash over the entire body, and treatment was discontinued. After several weeks the treatment was resumed and the rash recurred, indicating that the dermatitis was probably due to the drug.

PATIENTS TAKING FIRST COURSE ONLY

Case 1.—Female, aged 48. Tolerated treatment for a period of 8½ days, during which 510 grains of sulfanilamide were taken. The drug was discontinued because of an erythematous rash and acute lepra reaction, with fever. Death occurred 6 months later, of ovarian carcinoma.

Result: No improvement in the maculo-anesthetic lesions of leprosy.

Case 2.—Male, aged 30. Took 315 grains of sulfanilamide during 5 days of treatment. Fever and a severe lepra reaction developed; drug discontinued.

Result: No benefit; the patient believed that the disease was aggravated.

Case 3.—Male, aged 48. After 7½ days of treatment (436 grains of sulfanilamide), continuous drug fever as high as 39° C. necessitated discontinuation.

Result: No benefit; the patient refused to take the second course.

PATIENTS TAKING BOTH COURSES

Case 4.—Male, aged 31. First course begun on July 27, 1940, continued for 15 days (total drug, 660 grains). On the ninth day, the patient was hospitalized because of conjunctivitis and a mild febrile reaction (to 38.9° C.), which continued until the drug was discontinued.

Preliminary laboratory data: July 22, 1940, urinalysis, negative; nonprotein nitrogen, 33.3 mg. percent; urea clearance test, 64 percent of normal renal function. July 25, 1940: erythrocytes, 4,110,000; hemoglobin, 74 percent; leukocytes, 6,300; differential: stab cells 5, segmented cells 66, lymphocytes 23, monocytes 5, basophiles 1 percent; sedimentation test, 26 mm. in 1 hour.

Laboratory data during and after therapy: July 31, 1940: free sulfanilamide in blood, 10.5 mg. percent. August 6, 1940: erythrocytes, 3,540,000; leukocytes, 5,100; differential: stab cells 15, segmented cells 55, lymphocytes 21, monocytes 2, eosinophiles 6 percent. October 28, 1940: sedimentation test, 22 mm. in 1 hour. November 6, 1940: erythrocytes, 3,970,000; hemoglobin, 70 percent; leukocytes, 4,050; differential: stab cells 12, segmented cells 43, lymphocytes 25, monocytes 7, eosinophiles 13 percent.

Second course started November 7, 1940; drug taken for 43 days (total 1,410 grains). Severe neuritic pains in both legs experienced toward the end of the period, persisting until the drug was discontinued. Sulfanilamide concentration varied from 3.2 to 4.1 mg. Eosinophilia persisted, 13 percent. March 3, 1941: sedimentation test 26 mm.

Result: Ulcers of mouth and lips definitely improved. Lepromatous lesions of face less extensive. Macules over the body lighter in color and not as infiltrated as at first.

Case 5.—Male, aged 23. Completed first course of 47 days (1,620 grains) without toxic manifestations. Despite progressive anemia and severe neuritis, he also completed the second course of 70 days (2,160 grains).

Result: Some improvement of all leprous lesions.

Case 6.—Male, aged 29. Tolerated the first course for only 6½ days (375 grains). Neuritis and conjunctivitis supervened, and a severe toxic hepatitis developed. After a 3 months' rest he volunteered for the second course, which was discontinued after the third day when symptoms of hepatitis recurred.

Result: Improvement of general condition, with gain of 23 pounds in weight.

Case 7.—Male, aged 37. First course taken for 21 days (840 grains). Treatment stopped because of severe conjunctivitis and fever. The second course of 92 days (2,880 grains) was completed.

Result: Slight improvement in some leprous lesions.

Case 8.—Male, aged 50. First course completed (1,650 grains in 47 days) without evidence of toxic reactions.

Preliminary laboratory data: July 24, 1940: urinalysis, negative; nonprotein nitrogen, 42.8 mg.; urea clearance test, 44.3 percent. July 25, 1940: erythrocytes, 4,340,000; hemoglobin, 100; leukocytes, 7,500; differential: stab cells 3, segmented cells 64, lymphocytes 25, monocytes 3, eosinophiles 3, basophiles 2 percent; sedimentation test, 21 mm.

Laboratory data during and after therapy: July 30, 1940: free sulfanilamide, 16 mg. August 3, 1940: free sulfanilamide, 10.1 mg. September 3, 1940: erythrocytes, 3,450,000; leukocytes, 5,750; differential: stab cells 5, segmented cells 57, lymphocytes 26, monocytes 5, eosinophiles 5, basophiles 2 percent. October 28, 1940: sedimentation test, 9 mm.

The second course was also completed (2,160 grains in 70 days) without toxic symptoms. Sulfanilamide concentration varied between 4.4 and 7.0 mg. January 24, 1941: erythrocytes, 3,760,000; hemoglobin 82; leukocytes, 6,100; differential: stab cells 19, segmented cells 33, lymphocytes 31, monocytes 5, eosinophiles 12 percent.

Result: Improvement of leprous lesions noted, but since there had been a tendency towards improvement before therapy it cannot be credited to sulfanilamide alone.

Case 9.—Male, aged 35. First course tolerated for 7½ days (435 grains). Suspended because of continuous fever (39.5° C.) and severe lepra reaction. Second course continued for only 8 days (280 grains); discontinued for the same reasons—fever (40° C.) and acute lepra reaction.

Result: Progressive improvement occurred, but since this had started before instituting sulfanilamide therapy no credit can be given to the drug.

PATIENTS TAKING SECOND COURSE ONLY

Case 10.—Female, aged 35. Tolerated the drug for 76 days (2,400 grains) in spite of acute lepra reaction with fever (40° C.).

Result: No improvement.

Case 11.—Male, aged 20. Completed course of 88 days (1,970 grains). A mild lepra reaction occurred, with fever lasting 1 week; patient kept in bed and dosage temporarily decreased. Severe anemia with eosinophilia (10 percent) developed later.

Result: No apparent change in the lepromatous lesions.

Case 12.—Male, aged 23. After 27 days a generalized erythematous rash developed. It disappeared after the drug was discontinued, but recurred later when it was resumed. It was concluded probably to be a drug rash, and treatment was stopped. Total dosage, 1,005 grains.

Preliminary laboratory data: November 6, 1940: urinalysis, negative; nonprotein nitrogen, 34.6 mg.; erythrocytes, 4,360,000; hemoglobin, 82; leukocytes, 6,550; differential: stab cells 12, segmented cells 55, lymphocytes 29, monocytes 2, eosinophiles 2 percent; sedimentation test, 21 mm.

Laboratory data during and after therapy: November 19, 1940: free sulfanilamide, 6.4 mg. November 26, 1940: free sulfanilamide, 4.4 mg.; erythrocytes, 2,990,000; leukocytes, 10,600; juvenile cells 2, stab cells 16, segmented cells 61, lymphocytes, 15, monocytes 2, eosinophiles 2, basophiles 2 percent. March 20, 1940: sedimentation test, 25 mm.

Result: No improvement; disease stationary.

Case 13.—Male, aged 33. Took 820 grains during 34 days of treatment. Complications—chills and fever, conjunctivitis, epistaxis, and anemia caused discontinuance.

Result: A noticeable improvement in the lepromata of the face, with healing of some ulcerations of the nose.

Case 14.—Female, aged 23. Took the treatment for 40 days (1,260 grains). Drug fever developed and the drug was withheld for 4 days.

Result: Secondarily infected ulcerations of the legs healed, but the leprotic condition remained stationary or was aggravated.

Case 15.—Male, aged 51. Treatment given for 41 days (1,320 grains). There was an early, mild febrile reaction (38° C.) during which the dose was diminished. When fever recurred, treatment was stopped.

Result: No improvement.

Case 16.—Female, aged 32. Completed 84 days' treatment (2,580 grains). After the tenth day, chills and fever (39° C.) occurred and lasted several days; the dose was temporarily decreased.

Preliminary laboratory data: November 8, 1940: urinalysis, negative; nonprotein nitrogen, 30 mg.; erythrocytes, 4,020,000; hemoglobin, 70; leukocytes, 5,650; differential: stab cells 8, segmented cells 67, lymphocytes 23, monocytes 2 percent; sedimentation test, 23 mm.

Laboratory data during and after therapy: November 18, 1940: free sulfanilamide, 5.2 mg. November 25, 1940: free sulfanilamide, 6.2 mg. December 11, 1940: erythrocytes, 2,980,000; leukocytes, 7,450; differential: juvenile cells 5, stab cells 30, segmented cells 38, lymphocytes 25, monocytes 2 percent. March 22, 1941: sedimentation test, 29 mm.

Result: Slight improvement in the leprous lesions.

Case 17.—Female, aged 27. Tolerated 14 days of therapy (390 grains). High continuous fever developed (40° C.), followed by depression, delirium, and somnolence. There was hyperleukocytosis, and the condition became critical before fluids could be forced.

Result: The disease progressed, and the patient states that she has had more frequent lepra reactions than before.

Case 18.—Female, aged 40. Treated for 14 days (540 grains). Severe drug fever and marked depression required cessation of the treatment.

Result: No benefit.

Case 19.—Male, aged 40. Took treatment for 24 days (780 grains). A continuing drug fever (39° C.) led to discontinuing the drug.

Result: Progression was noted shortly after the treatment, new macules developing and some old ones becoming reactivated.

Case 20.—Female, aged 36. After 2 weeks, patient hospitalized for fever (40° C.). Altogether she took 21 days of treatment (720 grains).

Result: Unimproved.

SUMMARY OF LABORATORY DATA

No severe blood dyscrasia, such as granulocytopenia or severe hemolytic anemia, was experienced among the group treated.

Anemia.—Examinations of the average erythrocyte and hemoglobin levels showed, over a period of 80 days, a slow progressive drop in all cases. While in the majority of instances this anemia was mild, a significant decline up to 50 percent was not an unusual finding. In three cases, the drug was discontinued because of slow progressive anemia. The erythrocytes dropped from 4,100,000 to 1,970,000 per cmm. in 34 days in one of them, and from 3,790,000 to 2,100,000 in 40 days in another, while the hemoglobin fell from 84 to 48 percent in 78 days in the third.

Leukocytosis.—The persistence of leukocytosis of 12,000 per cmm. or more occurred in 14 of the cases, in whom 138 high counts were recorded. The figures averaged between 12,000 and 25,000. One case had a count of 50,200, and another had 40,300.

Leukopenia.—Leukopenia was considered to be present when the leukocyte count fell to a level below 5,000. Of the 20 patients, depression of the leukocytes below this level occurred in 6, for whom 27 low counts were recorded. In only 2 of the cases was the leukocyte depression noted more than once. The lowest count was 3,700.

Differential count.—Detailed examination of the leukocytes revealed significant changes in the neutrophiles and eosinophiles. All cases but one showed a shift to the left (Shilling's hemogram), whether they had leukopenia or leukocytosis. In 5 of those with leukopenia, myelocytes were noted once and juveniles in 17 instances.

Eosinophilia (5 to 23 percent) occurred in 12 of the cases and was found on from 1 to 19 occasions in each case, with a total of 65 times. Only 1 case had eosinophilia (7 percent) before treatment was started. On admittance the feces examination was negative for ova and worms, and no other cause for the eosinophilia was found. It is significant that it was this patient who developed the highest eosinophilia (23 percent) during the treatment.

Eosinophilia has not been reported in the literature as a result of treatment with sulfanilamide or related compounds. The reason for the increase in these cells in about one-half of the leprosy patients so treated is unknown. It is interesting that the two patients who developed severe drug rashes did not show eosinophilia, so that dermatitis was not the exciting factor. The acute leprous skin reactions noted in several of the cases also did not seem to account for it. Might not the appearance of eosinophiles in the blood stream in such large numbers in a chronic disease like leprosy indicate a favorable tissue reaction to the disease?

COMMENT ON COMPLICATIONS

On the whole, the toxic complications of sulfanilamide therapy in leprosy seemed to be more frequent and more severe than those reported in the literature as occurring in the treatment of other infectious diseases (*1*). The initiation of acute lepra reaction was not an unusual occurrence; it was noted in 9 of the cases treated. Drug fever was observed in 12 of the patients (60 percent). At times it was difficult to determine whether the patient's fever was due to toxicity of the drug or to the setting up of a lepra reaction by the drug. Neuritis was a complicating factor in 4 patients, whether caused by the drug directly or due to its stirring up of a lepra reaction in the nerve. Conjunctivitis was noted in 3 patients, and a drug dermatitis in 2. Finally, the changes produced in the blood pictures of all the patients, as noted above, seemed to be a rather unusual and severe type of reaction.

RESULTS OF THE TREATMENT

Of the entire group of 20 patients treated, 6 show some improvement of their leprous lesions. Two others show improvement, but they were improving when the treatment was started. One is probably stationary. The remaining 10 show probably slight progression of the disease. In 2 of the cases the sulfanilamide treatment definitely helped to clear up secondary infections. It is of interest in this connection that sulfanilamide therapy produced prompt improvement of pseudoerysipelas in 12 patients not included in this group. One patient of the treated group has since died of ovarian carcinoma, with no change in the leprotic condition.

CONCLUSIONS

Sulfanilamide therapy has proved effective in the treatment of secondary infections complicating leprosy, and as a help in the healing of secondarily infected leprous ulcerations.

Sulfanilamide cannot be regarded as a curative agent for leprous lesions, either of the macular or lepromatous type.

The significance of the development of eosinophilia during the course of sulfanilamide treatment is interesting and may be a fruitful field for future study.

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ANTIBACTERIAL ACTION OF SEVERAL SULFONAMIDE COMPOUNDS ON *HEMOPHILUS INFLUENZAE* TYPE b [1]

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Since the introduction of the use of sulfonamide compounds in the treatment of bacterial infections a number of reports have appeared concerning the use of sulfanilamide and sulfapyridine in the treatment of *Hemophilus influenzae* meningitis. In certain instances it seems that the compounds had a favorable influence on the course of the infections; in other instances they were without demonstrable influence. The early reports were largely of a single case or a small number of cases. These have been reviewed by Bilger and Haralambie (*1*)

[1] From the Division of Biologics Control, National Institute of Health. Received for publication April 1, 1942.

and Guyton (*2*). Some of the more recent reports contain the results of the treatment of larger numbers of cases.

Neter (*3*) reported 14 cases, stating "some were treated with antiserum, sulfanilamide, and sulfapyridine." Only one recovered. Aléman (*4*) wrote about the death of 7 children treated with sulfapyridine. All were under 2 years of age. On the other hand, Neal, Appelbaum, and Jackson (*5*) have reported the recovery of 10 of 25 patients (under 10 years of age) treated with sulfapyridine and its sodium salt; 4 received antiserum in addition to the drug. None of the recoveries, however, were of patients under 2 years of age. Lindsey, Rice, and Selinger (*6*), using sulfanilamide or sulfapyridine with antiserum, reported the recovery of 6 of 13 children. In addition to a highly potent rabbit antiserum, Alexander (*7*) used sulfanilamide and sulfapyridine (sulfathiazole in 2 cases) in the treatment of 26 cases. Sixteen recovered. No doubt much of her success can be attributed to the antiserum. Very recently Hoyne (*8*) reported the recovery of 5 patients under 2 years of age; 3 were treated with sulfapyridine, 1 with sulfanilamide, and 1 with both compounds.

The few experimental studies that have been reported indicate that sulfanilamide and sulfapyridine are to a certain extent antagonistic to *H. influenzae* and that sulfapyridine is more so than sulfanilamide.

In 1937 Long and Bliss (*9*) mentioned that a 1:10,000 concentration of sulfanilamide in 50 percent normal horse serum broth markedly inhibited the growth of *H. influenzae*. No details of the experiment were given. Povitsky (*10*), using neoprontosil and antiserum, observed that more mice survived following treatment with the two agents combined than with either alone. Pittman (*11*) reported that sulfapyridine was effective in protecting mice against an experimental infection of a virulent strain which was not type-specific. The percentage of survival varied directly with the dosage. Neter (*3*) observed that *H. influenzae* remained viable for 24 hours at 37°C. in spinal fluid containing sulfanilamide in concentrations of 5 to 15 mg. per 100 ml., while in the presence of corresponding amounts of sulfapyridine the bacteria were killed. Guyton (*2*), in a more extensive study, noted the effect of these two drugs on the growth of two strains of *H. influenzae* (one was not type-specific, the other was of Type b) in blood broth. He found that both drugs may produce an inhibitory or at times even a bactericidal effect, and that sulfapyridine in concentrations of 1 to 10 mg. per 100 ml. exerted a much greater effect than did sulfanilamide in the same concentrations.

In the present work we have used sulfanilamide, sulfapyridine, sulfathiazole, sulfadiazine,[2] and two other compounds, sulfanilyl sulfanilamide and para-nitrobenzoic acid, which have not been accepted for therapeutic use. A comparison of the action of each was made both on experimental infections in mice and on the growth of bacteria in culture medium. Observations on the susceptibility of cultures obtained from different patients were included. In addition, a study was made of the influence of treatment with (1) antiserum and sulfapyridine and (2) antiserum and sulfadiazine on infection in mice. Type b strains of *H. influenzae* were used throughout the investigation.

[2] The sulfadiazine was obtained through the courtesy of Calco Chemical Co.

MATERIALS AND METHODS

Cultures.—Six strains of Type b *H. influenzae*, numbered 571 to 576, were employed. All had been isolated directly from the spinal fluid of children suffering from meningitis before administration of either drug or antiserum. They were kept in defibrinated rabbit blood. Transfers were made into fresh blood every 2 weeks, the tubes incubated for 12 to 20 hours, and then stored in the ice box. Passages through mice were made frequently. The virulence of each strain was such that less than 10 bacteria suspended in mucin were usually lethal for 2 of 3 mice.

Preceding an experiment, 2 or 3 rapid transfers of the culture were made in Levinthal broth. A broth culture approximately 5 hours old was used in all experiments.

Mice.—Mice from a closely inbred colony of a white Swiss strain, weighing 15 to 19 grams, were employed. Those of one sex were used in a single experiment.

Mucin.—Two lots of mucin were used, one in a concentration of 3.5 percent and the other in a concentration of 5 percent. The necessity to use different concentrations emphasizes the variation in different lots which may be encountered. The solutions were prepared according to the method previously described (*12*). Alone they were apparently not toxic for mice.

Method of testing for antibacterial action in mice.—The procedure was similar to that described in a previous paper (*11*). That is, mice were given orally one dose of the drug followed by an intraperitoneal inoculation of the culture suspended in mucin. This method was selected in preference to giving the drug with food, because mice that are given intraperitoneal inoculations of mucin do not eat for a number of hours. The fallacies of this procedure will be pointed out later.

The sulfonamide compounds were suspended in 5 percent gum arabic with the desired dose in a volume of 0.4 ml. They were administered into the stomach by means of a silver eustachian catheter, child's size, attached to a tuberculin syringe.

Each of three groups of 10 mice were given a different amount of the compound under test. The amounts were progressively doubled, e. g., 2, 4, and 8 mg. The exact amount of each drug was selected so that if possible more than half of the mice receiving the smallest dose would succumb while more than half of those receiving the largest would survive. One hour after the administration of the drug the mice were inoculated intraperitoneally with 1 ml. of a 10^{-3} to 10^{-4} dilution of the culture prepared in mucin. This represented approximately 100,000 minimum fatal doses, yet it was sufficiently small not to cause death by toxicity. The bacilli injected with mucin rapidly multipled, and at death bacteria were generally recovered from the heart's blood.

Virulence titrations of each strain were carried out in each experiment. One ml. of the 10^{-8} dilution, containing approximately 5 to 7 bacteria, usually killed 2 of 3 mice.

The mice were kept under observation for 96 hours. Heart's blood cultures were made from all that died in order to ascertain whether they died from a *H. influenzae* infection or some other cause.

The results were calculated by the Reed-Muench (*13*) method to determine the amount of drug which theoretically would have protected 50 percent of the mice, i. e., the 50 percent endpoint of the drug.

Method of testing for antibacterial action in vitro.—A liver infusion medium, which MacLeod (*14*) claims does not inhibit the action of sulfonamides, was used for testing the antibacterial action of the compounds. To one preparation of the medium 1 percent casein hydrolysate was added. Influenza bacilli grew in the

medium without the addition of the accessory growth factors X (hematin) and V (di- or triphosphopyridine nucleotide). The growth-promoting property of the medium, however, was relatively unstable, even when the medium was kept in dark bottles and in the ice box.

A 1:500 dilution (10 mg. in 5 ml.) of the sulfonamide compound was prepared in distilled water or the medium, heated for 10 minutes in a boiling water bath, and then, while hot, 1 part was added to 19 parts of medium to give a 1: 10,000 dilution. Five ml. of each solution were placed in a test tube of 20 mm. diameter and inoculated with 0.1 ml. of a 2.5×10^{-5} dilution of a 5-hour culture. The liver-infusion medium was used as the diluent. The inoculum represented approximately 200 bacteria per ml. of medium.

The cultures were incubated for 24 hours in a water bath at 37.5° C. Since the marked fluorescence of the medium prevented the determination of growth by gross turbidity, the growth was determined by colony count. Tenfold serial dilutions (0.5 ml. in 4.5 ml. of diluent) of the culture were made in proteose peptone solution. One ml. of the culture and the respective dilutions was cultured in 10 ml. of nutrient 0.15 percent agar containing 0.1 ml. of Fildes' peptic digest of blood (*15*). The subcultures containing 10 or fewer bacteria showed isolated fluffy colonies.

EXPERIMENTAL

Comparison of antibacterial action of compounds on infection in mice.—In table 1 are summarized the results of 6 experiments on the

TABLE 1.—*Comparison of antibacterial action of 6 compounds on* H. influenzae *in mice*

Compound	50 percent endpoint of compound						Average activity ratio to sulfapyridine
	Dec. 18, 1940, No. 575	Mar. 13, 1941, No. 575	Mar. 19, 1941, No. 576	Apr. 3, 1941 No. 575	July 17, 1941, No. 572	July 22, 1941, No. 572	
	Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	
Sulfapyridine	4.35	6.4	2.7	2.83	4.32	2.7	1.0
Sulfathiazole	2.84(1.53)	3.5(1.83)	----------	----------	----------	2.32(1.17)	1.51
Sulfadiazine	<2.0	<0.5	----------	0.365(7.75)	0.5(8.64)	0.29(9.31)	8.57
Sulfanilamide	----------	----------	6.35(0.43)	----------	>10.0	>12.0	?
Sulfanilyl sulfanilamide	----------	----------	2.9 (0.93)	----------	9.0(0.48)	----------	0.70
Para-nitrobenzoic acid	----------	----------	<5.0	2.7 (1.05)	2.1(2.05)	----------	1.55

Figures in parentheses indicate activity ratio to sulfapyridine.

antibacterial action of the 6 compounds. In each experiment an estimate of the 50 percent endpoint of each drug was made, and where possible the activity of the compound in relation to sulfapyridine was determined. The strain and the size of inoculum varied in the separate experiments, hence varying endpoints for the same compound were obtained. However, the activity ratios of a single compound in the different experiments were fairly close in the majority of instances.

The activity of sulfadiazine was the highest, being 8.57 times that of sulfapyridine. The effectiveness of sulfathiazole and paranitrobenzoic acid was one and a half times that of sulfapyridine while

sulfanilyl sulfanilamide and sulfanilamide were less active than sulfapyridine. In only one of three experiments with sulfanilamide was the 50 percent endpoint obtained. In this particular experiment, culture No. 576 was used. It will be shown later that this strain was more sensitive to the action of all of the compounds than was any of the others employed.

Since these experiments did not take into account the blood levels of the compounds, the activity ratios which were obtained are not to be interpreted as the relative antibacterial values of the respective drugs.

TABLE 2.—*Variation in susceptibility of 6 cultural strains to sulfapyridine in vivo*

Strain	50 percent endpoint			Average
	Experiment No. 1 (Dec. 10, 1940)	Experiment No. 2 (June 11, 1941)	Experiment No. 3 (July 15, 1941)	
	Mg.	*Mg.*	*Mg.*	*Mg.*
571	2.65	3.0	----------	2.82
572	4.9	5.25	5.85	5.33
573	6.1	8.0	----------	7.05
574	<2.0	2.35	----------	2.0 (approx.)
575	5.2	5.7	5.22	5.37
576	<2.0	1.26	----------	<2.0

Dose of culture: 1 ml. of 10^{-4} dilution of culture.

Variation in susceptibility of bacterial strains.—The susceptibility of the 6 strains of *H. influenzae* to sulfapyridine *in vivo* are given in table 2. All strains were of similar virulence for mice. Six were used in the first two experiments and two in the third experiment. The amount of sulfapyridine required to protect 50 percent of the mice against the bacteria of a particular strain was in quite close agreement in the different experiments.

Strain No. 576 was found to be most sensitive to the action of sulfapyridine. In the first experiment, a sufficiently small dose of the drug was not given to determine the amount that would protect 50 percent of the mice. Smaller doses were given in the second experiment and the endpoint was found to be 1.26 mg. In the same experiment it was shown that 8 mg. was required to protect 50 percent of the mice against strain No. 573. In other words, slightly more than six times as much sulfapyridine was required to protect the mice against No. 573 as against No. 576. The susceptibility of the other four strains was between that of Nos. 576 and 573.

It should be noted that for each strain the amount of drug required to protect the mice in each experiment was practically the same. Since 6 months or more elapsed between the first and latter experiments, it is indicated that there was no change in the susceptibility of the 6 strains during the period of artificial cultivation.

The susceptibility of the 6 strains to the action of sulfadiazine *in vitro* was also studied. Although smaller amounts of the drug were required to protect the mice, the relative susceptibility or resistance of the different strains was the same as that obtained with sulfapyridine.

TABLE 3.—*Comparison of antibacterial action of 6 compounds on* H. influenzae *in cultural medium*

	Cultural strain					
	No. 571	No. 572	No. 573	No. 574	No. 575	No. 576
Original inoculum	600	270	230	----------	300	500
Sulfapyridine	120,000	4,000	1,400,000	----------	2,000	200
Sulfathiazole	200,000	400	40,000	----------	3,000	0
Sulfadiazine	70,000	2,000	160,000	----------	4,000	0
Medium control	50,000,000	500,000,000	>100,000,000	----------	14,000,000	200,000,000
Original inoculum	200	300	370	270	300	200
Sulfapyridine	300	100	10,000	60	20	10
Sulfathiazole	5,000	0	1,000	10	0	0
Sulfadiazine	1,000	0	1,000	30	0	0
Para-nitrobenzoic acid	0	0	0	0	0	0
Sulfanilyl sulfanilamide	4,000	20	20,000	100	0	0
Sulfanilamide	100,000	5,000,000	>100,000,000	4,000,000	300,000	10
Medium control	8,000,000	2,000,000	200,000,000	100,000,000	?	10,000,000

Liver infusion medium contained 1 percent casein hydrolysate.
Concentration of compound = 10 mg. percent.
The figures represent the number of bacteria per ml.
Cultures were incubated for 24 hours at 37.5° C.

Antibacterial action in culture medium.—The results of two tests on the antibacterial action of the compounds *in vitro* are given in table 3. In the first, 3 compounds and 5 cultural strains were used and in the second, 6 compounds and 6 cultural strains. All compounds were used in a concentration of 10 mg. percent.

It may be noted that there was less antibacterial action in the first than in the second experiment, but there was also less multiplication of the bacteria in the control medium. The same lot of medium was used for both, but the first was done when the medium was 9 days old and the second 5 days later. Apparently there had been some loss in the growth promoting properties of the medium during the intervening time.

The most active antibacterial action, obtained consistently, was observed with sulfadiazine and sulfathiazole. Similar results were observed with both. In their presence there was either marked inhibition in rate of multiplication or killing of the bacteria. No bacteria were recovered from the cultures of No. 576 in either experiment, nor of Nos. 572 and 576 in the second experiment.

The antibacterial action of sulfapyridine was probably next in order. In each instance there was either retardation in multiplication or reduction in number of bacteria. No culture, however, was sterilized.

The action of sulfanilyl sulfanilamide was about the same as that of sulfapyridine. In certain instances a greater number of bacteria were recovered, while in two instances no bacteria were recovered.

In contrast, sulfanilamide showed very little antibacterial action. The bacteria of all strains, except No. 576, increased in number the same or just slightly less than in the medium alone. In the culture of No. 576 the bacteria were reduced from 200 to 10 bacteria per ml.

With para-nitrobenzoic acid the results have not been consistent. In the second experiment of table 3 no bacteria of any strain were recovered, but in other experiments its antibacterial action was between that of sulfadiazine and sulfapyridine. Further work with para-nitrobenzoic acid is indicated.

TABLE 4.—*Protection of mice with combination of treatment with antiserum and sulfapyridine*

Agent	Dosage				Survival
Sulfapyridine and antiserum	5 mg. and 0.5 ml. of 1:500 dilution				70 percent.
	14S 6D				
Antiserum	0.5 ml. of dilution				50 percent endpoint 1:100 dilution (0.005 ml.).
	1:100	1:200	1:400	1:800	
	3S 3D	6D	6D	6D	
Sulfapyridine	5 mg.		10 mg.		50 percent endpoint 8.4 mg.
	9D		6S 3D		
Culture control	1 ml. of 10^{-3} dilution				20 percent.
	2S 8D				

Cultural strain No. 571.
All mice were inoculated with 1 ml. of a 10^{-3} dilution of culture.

Treatment with both antiserum and sulfonamides.—In the following experiments the influence of sulfapyridine and sulfadiazine on the protection of mice that were also treated with antiserum was observed. The results of one experiment with sulfapyridine are given in table 4.

Twenty mice were given, simultaneously, 5 mg. of sulfapyridine orally and 0.001 ml. of unrefined rabbit antiserum intraperitoneally, then 1 hour later 1 ml. of the culture, suspended in mucin, intraperitoneally. Fourteen, or 70 percent, of the mice survived. The protective activity of the serum alone was determined by inoculating four groups of 6 mice each with 0.5 ml. of dilutions ranging from 1:100 to 1:800. One-half of the mice that received the 1:100 dilution (0.005 ml.) survived. The action of the compound alone was determined by inoculating two groups of 10 mice each with 5 mg. and 10 mg., respectively. All died which received the smaller dose; the calculated 50 percent endpoint was 8.4 mg. Since 5 mg. of sulfapyri-

dine failed to protect any mice and 0.005 ml. of serum protected only half of the mice, it appears that the survival of 70 percent of the mice which received the combined treatment of this same amount of compound and one-fifth this amount of serum (0.001 ml.) was not due merely to an additive effect of the two agents.

TABLE 5.—*Protection of mice with combination of treatment with antiserum and sulfadiazine*

Agent	Dosage				Survival
Sulfadiazine and antiserum	0.5 mg. and 0.5 ml. of 1:500 dilution				100 percent.
	20S				
Antiserum	0.5 ml. of dilution				50 percent endpoint 1:155 dilution (0.0032 ml.).
	1:50	1:100	1:200	1:400	
	4S 1D	5S 1D	2S 4D	1S 5D	
Sulfadiazine	0.5 mg.		1.0 mg.		50 percent endpoint 0.5 mg.
	6S 4D		8S 2D		
Culture control	1 ml. of 10^{-3} dilution				None.
	10D				

Cultural strain No. 575.

In table 5 are given the results of a similar experiment with sulfadiazine. In this instance, all mice survived which were treated with both sulfadiazine and antiserum. Five-tenths mg. and 0.001 ml. of the respective agents were used. The 50 percent endpoint of the drug was 0.5 mg. and of the serum it was 0.0032 ml. As with sulfapyridine, the protection of the mice with the combined treatment seems to be greater than would be expected from the additive effect of the two agents.

TABLE 6.—*Variation in susceptibility of 3 strains of* H. influenzae *to antiserum*

		Strain [1]		
		No. 571	No. 575	No. 576
Dilution of serum [2]	1:25			10 S
	1:50		6 S 4 D	3 S 7 D
	1:100	6 S 4 D	4 S 6 D	2 S 8 D
	1:200	8 S 2 D	10 D	
	1:400	5 S 5 D		
Calculated 50 percent endpoint		1:280	1:71	1:45
Virulence titration	10^{-6}	3 D	3 D	3 D
	10^{-7}	2 S 1 D	2 S 1 D	3 D [3]
	10^{-8}	2 S 1 D	3 D	1 S 2 D

[1] Inoculum = 1 ml. of 10^{-3} dilution in mucin.
[2] Inoculum = 0.5 ml.
[3] One death was not specific.

Variation in susceptibility of different bacterial strains to antiserum.—In tables 2 and 3 it was demonstrated that different strains of Type b *H. influenzae* were not uniform in susceptibility to the sulfonamide compounds. In table 6 it is shown that they, likewise, varied in sensitivity to the action of antiserum *in vivo*. Susceptibility to serum, however, did not parallel sensitivity to the compounds.

In the experiment recorded in table 6, three strains, Nos. 571, 575, and 576, were employed. The antiserum was the same as was used in the preceding experiments. The calculated 50 percent endpoints of the serum against the respective strains were 1:280, 1:71, and 1:45. A repetition of this experiment gave similar results. No. 576 was the most resistant strain to the action of the antiserum whereas with the compounds it was found, both *in vivo* and *in vitro*, to be the least resistant of the 6 strains.

DISCUSSION

In the present study it has been shown that, under the conditions of our experiments, sulfadiazine, sulfathiazole, and sulfapyridine are capable of exerting antibacterial action against Type b *H. influenzae* and that, relatively, sulfanilamide has little power. In the experiments with mice, about one-eighth as much of sulfadiazine and two-thirds as much of sulfathiazole as of sulfapyridine was required to protect 50 percent of the mice. These experiments, however, did not take into consideration either the rate of absorption and excretion or the blood level of the compounds. Since it has been shown by others (*16*, *17*) that with the same dosage higher blood levels are obtained with sulfadiazine than with sulfathiazole or sulfapyridine, it may be concluded that at least part of the variation was due to differences in concentrations in the blood. Furthermore, in culture medium these wide differences in activity were not observed. However, it was observed that sulfadiazine and sulfathiazole were slightly more active than sulfapyridine. The former two effected similar action.

Sulfanilamide, both *in vivo* and *in vitro*, was found to have very little antibacterial activity against 5 of the 6 strains tested. Against the sixth strain it was active.

Differences in strain sensitiveness or resistance were also observed when sulfadiazine, sulfathiazole, and sulfapyridine were used. The only strain that was sensitive to the action of sulfanilamide was the one most sensitive to the action of the other compounds. Only one-sixth as much sulfapyridine was required to protect mice against this strain as against the most resistant one. This unequal susceptibility of different strains suggests that the equivocal clinical results that have been obtained may be in part due to variations in susceptibility of the infecting organisms.

In the work with the two remaining compounds, sulfanilyl sulfanilamide and para-nitrobenzoic acid, it was found that the former probably was less active than sulfapyridine; with the latter, antibacterial action *in vivo* was about the same as with sulfathiazole, but *in vitro* the results were irregular. In some instances its activity was comparable to that of sulfathiazole, while in others it was more marked. Although this compound does have a certain amount of antibacterial power, its toxicity excludes therapeutic application. Frequently 10 mg. caused death in 16 to 20 gm. mice.

From the results of our experiments, it is suggested that of the 6 compounds studied, sulfadiazine and sulfathiazole are probably the most active against Type b *H. influenzae*. If the early reports (*16*, *18*, *19*) that sulfadiazine is less toxic than sulfapyridine or sulfathiazole are substantiated, then the use of sulfadiazine might be preferable in the treatment of infections caused by Type b *H. influenzae*.

On the other hand, sulfathiazole may be more active than it was formerly thought to be. When the use of sulfathiazole was introduced, several workers (*20*, *21*) advised against its use for the treatment of meningitis because of its low absorption in the spinal fluid. Nevertheless, it has been used in the treatment of meningoccocus meningitis with favorable results (*22*). Recently, Davis (*23*) presented data in a preliminary report which suggest that the level of sulfonamides in the spinal fluid is approximately the same as the level of the "free" or active drug in the blood plasma. In the case of sulfathiazole, he found that in the presence of human plasma 75 percent of this compound was bound and only 25 percent of it was free. The latter amount corresponds to the relative value usually found in the spinal fluid. If Davis' explanation is correct, then a comparison of the relative blood levels of sulfonamides may not give a true index of the relative values of the compounds.

In addition to our observations on the protection of mice with the sulfonamides alone, it was found that the combined treatment of either sulfadiazine or sulfapyridine with specific antiserum protected more mice than could be expected from the additive effect of the two agents.

Furthermore, it was found that different strains of bacteria varied considerably in sensitivity both to the action of drugs and of antiserum and that there was no correlation in sensitivity. For example, the culture which was most sensitive to drugs was the one most resistant to the action of antiserum. It therefore seems that by using combined treatment the chances of administering an effective agent are greatly increased. It was mentioned in the introduction that the combined treatment has been used with favorable results by Lindsey, Rice, and Selinger (*6*), and by Alexander (*7*). According to the latter, in

the present state of our knowledge a combination of antibody and chemotherapy offers the best prognosis of *H. influenzae* meningitis.

SUMMARY

1. Using a single dose, in protecting mice against Type b *Hemophilus influenzae* infection, sulfadiazine was found to be the most effective; sulfathiazole and para-nitrobenzoic acid of similar activity were slightly better than sulfapyridine; sulfanilyl sulfanilamide was less active than sulfapyridine. Antibacterial action of sulfanilamide was demonstrable against only 1 of 6 strains.

2. *In vitro*, sulfadiazine, sulfathiazole, sulfapyridine, and sulfanilyl sulfanilamide either markedly retarded growth or killed the bacteria. The action of sulfadiazine and sulfathiazole was similar and slightly better than that of sulfapyridine; the action of sulfanilyl sulfanilamide was fairly close to that of sulfapyridine. In the presence of sulfanilamide the amount of growth was equal to or only slightly less than that in the control medium, except with one strain. In this instance, the bacteria were actually diminished in number. The results with para-nitrobenzoic acid were inconclusive.

3. Different bacterial strains with apparently the same virulence for mice showed marked variation in susceptibility to the same compound both *in vivo* and *in vitro*. In mice, slightly more than six times as much sulfapyridine was required to protect against the most resistant strain as compared with the least resistant one.

4. The results of treatment with a combination of sulfapyridine or of sulfadiazine with specific antiserum seemed to be better than an additive effect of the two agents.

5. Different bacterial strains also varied in susceptibility to the action of antiserum. This sensitivity was not correlated with drug sensitivity.

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DEATHS DURING WEEK ENDED NOVEMBER 28, 1942

[From the Weekly Mortality Index, issued by the Bureau of the Census, Department of Commerce]

	Week ended Nov. 28, 1942	Corresponding week 1941
Data from 87 cities of the United States:		
Total deaths	8,434	8,332
Average for 3 prior years	8,378	
Total deaths, first 47 weeks of year	390,191	388,198
Deaths per 1,000 population, first 47 weeks of year, annual rate	11.7	11.6
Deaths under 1 year of age	602	561
Average for 3 prior years	542	
Deaths under 1 year of age, first 47 weeks of year	26,977	24,660
Data from industrial insurance companies:		
Policies in force	65,271,636	64,683,252
Number of death claims	9,820	12,684
Death claims per 1,000 policies in force, annual rate	7.8	10.2
Death claims per 1,000 policies, first 47 weeks of year, annual rate	9.1	9.4

PREVALENCE OF DISEASE

No health department, State or local, can effectively prevent or control disease without knowledge of when, where, and under what conditions cases are occurring

UNITED STATES

REPORTS FROM STATES FOR WEEK ENDED DECEMBER 5, 1942

Summary

No unusual incidence of the communicable diseases was reported during the week. Of the 9 common communicable diseases included in the table on the following pages, the current incidence of only one—meningococcus meningitis—is above the 5-year (1937–41) median.

A total of 88 cases of meningococcus meningitis was reported, as compared with 93 cases for the preceding week, and a 5-year median of 35 for the corresponding week. The largest numbers of cases were reported in the Middle Atlantic (27), New England (16), and South Atlantic States (14). New York reported the largest number (15, 11 in New York City) for any State. Rhode Island reported 8 and Pennsylvania and Virginia 7 each. No other State reported more than 5 cases.

The number of cases of poliomyelitis increased from 69 to 79, of which 19 cases occurred in Texas and 18 in California. New York reported 7 cases. No other State reported more than 3 cases.

The incidence of influenza increased slightly, from 1,854 cases last week to 1,928 currently. Of the total, Texas reported 769, South Carolina 322, and Virginia 187, or 66 percent in these three States.

A total of 64 cases of endemic typhus fever was reported, as compared with 63 for the preceding week. To date, 3,419 cases have been reported, as compared with 2,998 for the entire year 1939 and 2,787 in 1941, the years in which the largest numbers of cases had previously been recorded.

Plague infection was reported in two specimens of fleas and in tissue from one rat in Tacoma, Wash.

Other diseases include 2 cases of anthrax (in Pennsylvania), 18 cases of smallpox (7 in Indiana and 4 in Kansas), and 17 cases of tularemia (9 in the East North Central States.)

The death rate for the current week in 88 large cities in the United States is 13.5 per 1,000 population, as compared with 11.9 for both the preceding week and the 3-year (1939–41) average. Excluding the mortality resulting from the Boston fire, the current rate is 12.8, which is still 7.5 percent above the rate for the preceding week and the 3-year average.

Telegraphic morbidity reports from State health officers for the week ended Dec. 5, 1942, and comparison with corresponding week of 1941 and 5-year median

In these tables a zero indicates a definite report, while leaders imply that, although none were reported, cases may have occurred.

Division and State	Diphtheria			Influenza			Measles			Meningitis, meningococcus		
	Week ended		Median 1937–41	Week ended		Median 1937–41	Week ended		Median 1937–41	Week ended		Median 1937–41
	Dec. 5, 1942	Dec. 6, 1941		Dec. 5, 1942	Dec. 6, 1941		Dec. 5, 1942	Dec. 6, 1941		Dec. 5, 1942	Dec. 6, 1941	
NEW ENG.												
Maine	0	0	1	-----	1	2	1	352	40	2	0	0
New Hampshire	0	0	0	-----	-----	-----	101	8	3	0	0	0
Vermont	0	2	0	-----	-----	-----	157	0	24	0	0	0
Massachusetts	3	2	6	-----	-----	-----	370	141	211	5	5	1
Rhode Island	2	4	0	1	-----	-----	1	2	0	8	0	0
Connecticut	0	1	2	8	1	2	201	59	33	1	1	0
MID. ATL.												
New York	18	16	22	[1] 11	[1] 8	[1] 8	227	295	373	15	3	3
New Jersey	5	4	12	14	7	10	31	17	21	5	1	1
Pennsylvania	23	7	30	6	-----	-----	760	495	495	7	5	5
E. NO. CEN.												
Ohio	24	19	41	9	14	14	22	36	45	3	0	1
Indiana	9	17	25	16	47	32	44	17	17	0	1	1
Illinois	24	34	39	8	14	14	31	24	28	3	0	0
Michigan [2]	10	3	10	1	-----	3	56	39	156	0	1	0
Wisconsin	0	1	2	16	4	47	128	0	82	0	2	0
W. NO. CEN.												
Minnesota	9	5	4	2	1	1	7	33	33	0	0	0
Iowa	2	2	4	-----	2	1	21	32	37	0	1	1
Missouri	4	6	15	5	14	14	6	12	9	3	0	0
North Dakota	0	1	2	16	10	10	1	88	2	0	0	0
South Dakota	12	8	1	-----	-----	-----	29	1	1	0	2	0
Nebraska	7	2	4	5	-----	-----	59	9	2	0	0	0
Kansas	15	5	6	2	8	9	27	110	32	0	0	0
SO. ATL.												
Delaware	0	0	0	-----	1	-----	3	2	3	0	0	0
Maryland [2]	6	12	11	15	3	4	8	85	8	4	4	1
Dist. of Col	1	0	2	2	2	1	4	4	2	1	0	0
Virginia	23	40	38	187	250	145	13	202	69	7	0	0
West Virginia	10	12	15	14	6	6	4	230	11	0	2	2
North Carolina	37	70	64	3	6	6	5	267	141	0	0	2
South Carolina	16	19	19	322	409	371	3	29	20	0	0	1
Georgia	10	19	21	14	40	63	0	27	27	1	0	0
Florida	4	4	4	2	10	10	1	2	4	1	0	0
E. SO. CEN.												
Kentucky	9	16	16	3	6	12	37	175	76	2	2	2
Tennessee	4	13	13	29	30	40	8	51	21	1	1	1
Alabama	16	35	31	58	65	65	5	62	18	1	0	1
Mississippi [2]	10	18	17	-----	-----	-----	-----	-----	-----	0	1	1
W. SO. CEN.												
Arkansas	14	22	16	73	117	94	8	79	12	0	0	0
Louisiana	6	9	16	5	8	8	3	3	1	2	1	1
Oklahoma	17	17	21	60	104	88	3	6	6	0	1	0
Texas	45	83	57	769	1,245	354	16	250	64	3	1	1
MOUNTAIN												
Montana	2	2	2	-----	10	10	15	52	10	0	0	0
Idaho	1	0	2	1	-----	1	143	8	9	0	0	0
Wyoming	0	0	0	76	2	2	27	3	3	0	0	0
Colorado	10	13	10	50	49	28	10	41	41	1	0	0
New Mexico	2	3	3	1	-----	2	4	12	12	2	0	0
Arizona	6	1	4	68	127	121	1	51	2	0	0	0
Utah [2]	7	0	0	3	10	11	362	29	29	1	0	0
Nevada	0	0	-----	2	-----	-----	33	3	-----	1	0	-----
PACIFIC												
Washington	8	0	3	-----	2	-----	425	6	34	2	0	0
Oregon	1	2	2	29	16	17	235	40	15	2	0	1
California	29	20	39	22	93	63	61	509	160	4	0	1
Total	461	569	718	1,928	2,742	2,742	3,717	3,998	3,998	88	35	35
48 weeks	14,312	15,358	21,731	98,419	511,878	191,873	487,003	847,605	363,819	3,284	1,884	1,884

See footnotes at end of table.

Telegraphic morbidity reports from State health officers for the week ended Dec. 5, 1942, and comparison with corresponding week of 1941 and 5-year median—Con.

Division and State	Poliomyelitis			Scarlet fever			Smallpox			Typhoid and paratyphoid fever		
	Week ended		Median 1937–41	Week ended		Median 1937–41	Week ended		Median 1937–41	Week ended		Median 1937–41
	Dec. 5, 1942	Dec. 6, 1941		Dec. 5, 1942	Dec. 6, 1941		Dec. 5, 1942	Dec. 6, 1941		Dec. 5, 1942	Dec. 6, 1941	
NEW ENG.												
Maine	1	6	0	1	18	18	0	0	0	1	2	2
New Hampshire	0	1	0	7	3	4	0	0	0	0	0	0
Vermont	1	0	0	1	7	7	0	0	0	0	0	0
Massachusetts	0	5	1	285	268	140	0	0	0	1	3	1
Rhode Island	0	0	0	7	10	10	0	0	0	1	0	0
Connecticut	0	0	0	39	39	39	0	0	0	0	1	1
MID. ATL.												
New York	7	16	4	225	273	?73	0	0	0	5	6	8
New Jersey	3	4	2	65	95	97	0	0	0	2	5	4
Pennsylvania	3	2	2	162	137	267	0	0	0	3	6	6
E. NO. CEN.												
Ohio	2	5	3	238	259	295	0	0	1	4	18	7
Indiana	1	0	0	43	99	153	7	10	10	1	1	1
Illinois	3	4	4	217	196	330	1	0	1	1	1	6
Michigan [2]	1	0	2	91	141	281	0	1	4	2	7	5
Wisconsin	1	1	3	135	140	151	0	1	2	0	1	0
W. NO. CEN.												
Minnesota	0	2	2	73	89	117	0	1	12	0	0	0
Iowa	0	0	1	48	42	86	1	4	6	1	0	1
Missouri	1	0	1	54	49	66	1	3	7	3	2	4
North Dakota	0	1	0	10	21	21	0	0	1	0	1	0
South Dakota	1	1	1	29	50	28	0	0	0	0	1	0
Nebraska	3	0	0	15	13	33	0	0	0	2	1	1
Kansas	3	1	0	53	88	100	4	1	1	1	1	1
SO. ATL.												
Delaware	0	0	0	17	22	22	0	0	0	0	0	0
Maryland [2]	0	1	0	36	61	52	0	0	0	3	4	4
Dist. of Col	0	0	0	33	15	15	0	0	0	1	1	0
Virginia	1	3	1	58	97	52	0	0	0	3	5	6
West Virginia	0	1	1	46	51	64	0	1	0	1	5	3
North Carolina	1	1	1	111	99	99	0	0	0	0	6	2
South Carolina	0	3	0	6	17	17	0	0	0	0	3	2
Georgia	0	2	1	43	42	34	0	0	0	3	5	5
Florida	0	0	0	13	7	5	0	0	0	2	2	2
E. SO. CEN.												
Kentucky	1	2	2	62	100	96	1	0	0	0	9	4
Tennessee	0	12	0	58	58	58	0	1	0	3	4	4
Alabama	1	8	2	44	42	35	0	0	0	1	0	1
Mississippi [2]	1	3	1	14	25	15	1	0	0	3	3	1
W. SO. CEN.												
Arkansas	0	1	1	14	5	17	2	1	1	3	7	6
Louisiana	0	3	1	19	14	14	0	0	0	3	9	9
Oklahoma	0	2	1	20	24	24	0	1	1	4	2	4
Texas	19	2	2	40	67	67	0	0	0	5	8	10
MOUNTAIN												
Montana	0	0	0	10	48	31	0	0	1	2	0	1
Idaho	0	0	0	8	3	12	0	0	0	0	0	1
Wyoming	0	0	0	2	8	8	0	0	0	0	0	0
Colorado	0	0	1	25	28	41	0	0	0	4	0	1
New Mexico	1	0	0	17	12	17	0	0	0	2	3	7
Arizona	1	0	0	6	4	4	0	0	0	1	0	0
Utah [2]	3	0	0	27	22	24	0	0	0	1	0	0
Nevada	0	0	------	0	2	--------	0	0	------	0	0	------
PACIFIC												
Washington	0	2	2	27	25	46	0	0	1	0	0	1
Oregon	1	2	1	10	20	24	0	0	1	0	2	2
California	18	2	5	153	136	176	0	0	0	5	5	5
Total	79	99	91	2,717	3,091	3,880	18	25	50	78	140	140
48 weeks	3,981	8,899	8,899	115,871	116,073	147,380	737	1,286	9,161	6,453	8,023	12,265

See footnotes at end of table.

Telegraphic morbidity reports from State health officers for the week ended Dec. 5, 1942—Con.

Division and State	Whooping cough		Week ended Dec. 5, 1942								
	Week ended		Anthrax	Dysentery			Encephalitis, infectious	Leprosy	Rocky Mt. spotted fever	Tularemia	Typhus fever
	Dec. 5, 1942	Dec. 6, 1941		Amebic	Bacillary	Unspecified					
NEW ENG.											
Maine	73	36	0	0	0	0	1	0	0	0	0
New Hampshire	14	19	0	0	0	0	0	0	0	0	0
Vermont	46	13	0	0	0	1	0	0	0	0	0
Massachusetts	258	218	0	0	2	0	1	0	0	0	0
Rhode Island	39	36	0	0	0	0	0	0	0	0	0
Connecticut	107	58	0	0	0	0	0	0	0	0	0
MID. ATL.											
New York	436	675	0	2	15	0	2	0	0	0	0
New Jersey	270	259	0	0	0	0	0	0	0	1	0
Pennsylvania	371	165	2	0	0	0	0	0	0	1	0
E. NO. CEN.											
Ohio	183	229	0	0	0	0	0	0	0	3	0
Indiana	15	17	0	0	0	0	0	0	0	1	0
Illinois	195	281	0	2	0	0	0	0	0	3	0
Michigan [2]	250	282	0	0	4	0	0	0	0	0	0
Wisconsin	195	354	0	0	0	0	0	0	0	2	0
W. NO. CEN.											
Minnesota	47	72	0	0	0	0	0	0	0	0	0
Iowa	31	22	0	0	0	0	0	0	0	0	0
Missouri	12	9	0	0	0	1	0	0	0	0	0
North Dakota	19	10	0	0	0	0	0	0	0	0	0
South Dakota	7	9	0	0	0	0	0	0	0	0	0
Nebraska	6	2	0	0	0	0	0	0	0	0	0
Kansas	29	84	0	0	0	0	0	0	0	0	0
SO. ATL.											
Delaware	6	11	0	0	0	0	0	0	0	0	0
Maryland [2]	109	28	0	0	0	12	1	0	0	0	0
Dist. of Col	13	20	0	0	0	0	0	0	0	0	0
Virginia	34	85	0	0	0	20	0	0	0	0	1
West Virginia	33	40	0	0	0	0	0	0	0	0	0
North Carolina	55	142	0	0	0	0	0	0	0	0	3
South Carolina	13	44	0	0	0	0	0	0	0	0	3
Georgia	15	7	0	0	6	0	0	0	0	1	20
Florida	18	10	0	1	1	0	0	0	0	0	3
E. SO. CEN.											
Kentucky	17	90	0	0	0	0	0	0	0	1	0
Tennessee	42	43	0	0	0	0	0	0	0	0	0
Alabama	31	21	0	0	0	0	0	0	0	0	3
Mississippi [2]	----	----	0	0	0	0	0	0	0	0	1
W. SO. CEN.											
Arkansas	22	30	0	3	1	0	0	0	0	0	1
Louisiana	3	1	0	4	1	0	0	0	0	1	6
Oklahoma	5	4	0	0	0	0	0	0	0	0	0
Texas	173	120	0	2	69	0	0	0	0	0	23
MOUNTAIN											
Montana	20	41	0	0	0	0	0	0	0	0	0
Idaho	5	12	0	0	0	0	0	0	0	0	0
Wyoming	2	13	0	0	0	0	0	0	0	0	0
Colorado	11	50	0	0	0	0	1	0	0	1	0
New Mexico	16	32	0	0	1	0	0	0	0	0	
Arizona	9	11	0	0	0	47	0	0	0	0	0
Utah [2]	11	31	0	0	0	0	0	0	0	1	0
Nevada	0	8	0	0	0	0	0	0	0	0	0
PACIFIC											
Washington	26	160	0	0	2	0	0	0	0	0	0
Oregon	8	30	0	1	0	0	0	0	0	0	0
California	220	192	0	5	9	0	2	0	0	1	0
Total	3,525	4,126	2	20	111	81	8	0	0	17	64
48 weeks	165,897	195,672	----	----	----	----	----	----	----	----	----

[1] New York City only.
[2] Period ended earlier than Saturday.

WEEKLY REPORTS FROM CITIES

City reports for week ended Nov. 21, 1942

This table lists the reports from 87 cities of more than 10,000 population distributed throughout the United States, and represents a cross section of the current urban incidence of the diseases included in the table.

	Diphtheria cases	Encephalitis, infectious, cases	Influenza		Measles cases	Meningitis, meningococcus, cases	Pneumonia deaths	Poliomyelitis cases	Scarlet fever cases	Smallpox cases	Typhoid and paratyphoid fever cases	Whooping cough cases
			Cases	Deaths								
Baltimore, Md	6	0	1	0	1	3	17	0	7	0	0	86
Barre, Vt	0	0	------	0	63	0	0	0	0	0	0	0
Billings, Mont	0	0	------	0	0	0	1	0	1	0	0	4
Birmingham, Ala	0	1	7	0	0	0	2	0	2	0	0	0
Boise, Idaho	0	0	------	0	1	0	1	0	0	0	0	0
Boston, Mass	0	0	------	0	11	4	19	0	76	0	0	41
Bridgeport, Conn	1	0	1	1	0	0	6	0	3	0	0	1
Brunswick, Ga	0	0	------	0	0	0	0	0	0	0	0	0
Buffalo, N. Y	0	0	------	2	18	0	11	0	8	0	0	14
Camden, N. J	0	0	------	1	1	0	1	0	4	0	0	13
Charleston, S. C	0		22	1	0	0	4	0	0	0	0	0
Charleston, W. Va	0	0	------	0	0	0	0	0	2	0	0	0
Chicago, Ill	17	0	1	2	14	1	27	3	48	0	0	78
Cincinnati, Ohio	5	0	1	0	6	0	1	0	31	0	0	6
Cleveland, Ohio	3	0	6	1	2	0	3	0	40	0	0	69
Columbus, Ohio	1	0	1	1	1	0	3	0	31	0	0	8
Concord, N. H	0	0	------	0	1	0	0	0	2	0	0	0
Cumberland, Md	0	0	------	0	0	0	1	0	0	0	0	0
Dallas, Tex	0	0	1	1	0	0	4	0	3	0	0	1
Denver, Colo	5	0	12	1	4	1	4	0	6	0	0	4
Detroit, Mich	1	0	------	1	11	1	10	0	52	0	1	118
Duluth, Minn	0	0	------	0	0	0	1	0	3	0	0	1
Fall River, Mass	0	0	------	0	0	0	1	0	10	0	0	4
Fargo, N. Dak	0	0	------	0	0	0	0	0	0	0	0	0
Flint, Mich	0	0	------	0	0	0	4	0	7	0	0	13
Fort Wayne, Ind	0	0	------	0	0	0	0	0	0	0	0	0
Frederick, Md	0	0	------	0	0	0	0	0	0	0	0	0
Galveston, Tex	0	0	------	0	0	0	4	0	1	0	0	0
Grand Rapids, Mich	0	0	1	0	0	0	1	0	1	0	0	6
Great Falls, Mont	0	0	------	0	0	0	0	0	2	0	0	2
Hartford, Conn	1	0	------	0	1	0	5	0	1	0	0	15
Helena, Mont	0	0	------	0	1	0	0	0	0	0	0	0
Houston, Tex	3	0	------	0	0	0	2	0	2	0	0	0
Indianapolis, Ind	0	0	------	0	3	0	10	0	11	1	0	8
Kansas City, Mo	0	0	------	1	1	0	2	0	24	0	0	1
Kenosha, Wis	0	0	------	0	0	0	0	0	6	0	0	0
Little Rock, Ark	0	0	------	0	0	0	2	0	1	0	0	1
Los Angeles, Calif	1	0	11	0	4	0	16	13	20	0	0	22
Lynchburg, Va	1	0	------	0	0	0	0	0	0	0	0	0
Memphis, Tenn	0	0	1	2	1	0	12	0	4	0	1	13
Milwaukee, Wis	0	0	1	1	48	0	5	0	66	0	0	18
Minneapolis, Minn	1	0	------	0	0	0	4	3	14	0	1	9
Missoula, Mont	0	0	------	0	0	0	1	0	0	0	1	0
Mobile, Ala	0	0	------	0	0	0	2	0	3	0	0	0
Nashville, Tenn	0	0	------	0	1	0	4	0	0	0	0	4
Newark, N. J	0	0	2	1	5	1	4	0	6	0	1	18
New Haven, Conn	0	0	------	1	0	0	2	0	3	0	0	17
New Orleans, La	0	0	1	0	2	0	13	0	1	0	0	1
New York, N. Y	12	0	16	0	10	9	57	1	138	0	3	128
Omaha, Nebr	0	0	------	0	0	0	4	0	1	0	0	0
Philadelphia, Pa	5	0	6	3	357	1	32	0	40	0	1	12
Pittsburgh, Pa	3	0	3	4	0	0	7	1	4	0	0	23
Portland, Me	0	0	1	1	0	0	0	0	3	0	0	25
Providence, R. I	0	0	------	0	0	0	0	0	0	0	0	1

City reports for week ended Nov. 21, 1942—Continued

	Diphtheria cases	Encephalitis, infectious, cases	Influenza		Measles cases	Meningitis, meningococcus, cases	Pneumonia deaths	Poliomyelitis cases	Scarlet fever cases	Smallpox cases	Typhoid and paratyphoid fever cases	Whooping cough cases
			Cases	Deaths								
Pueblo, Colo	0	0	------	0	0	0	1	0	3	0	0	0
Racine, Wis	0	0	------	0	4	0	1	0	11	0	0	2
Reading, Pa	0	0	------	0	1	0	1	0	2	0	0	23
Richmond, Va	1	0	1	1	0	0	0	0	6	0	1	1
Roanoke, Va	1	0	------	0	2	0	0	0	3	0	0	0
Rochester, N. Y	0	0	------	0	1	0	5	0	5	0	0	16
Sacramento, Calif	3	0	------	0	0	0	1	0	4	0	1	4
Saint Joseph, Mo	0	0	------	0	0	0	1	0	0	0	0	0
Saint Louis, Mo	0	0	------	1	1	0	9	1	11	0	0	1
Saint Paul, Minn	0	0	------	0	0	0	3	0	6	0	0	27
Salt Lake City, Utah	0	0	------	0	69	0	0	0	7	0	0	6
San Antonio, Tex	3	0	1	1	0	0	1	10	1	0	0	3
San Francisco, Calif	1	0	------	0	10	1	11	0	5	0	0	10
Savannah, Ga	0	0	1	0	0	0	1	0	0	0	0	0
Seattle, Wash	0	0	------	0	11	0	6	2	0	0	1	5
Shreveport, La	3	0	------	0	0	0	9	0	2	0	0	0
South Bend, Ind	0	0	------	0	1	0	0	0	1	0	0	1
Spokane, Wash	0	0	------	0	27	0	0	1	1	0	0	0
Springfield, Ill	0	0	------	0	0	0	3	0	5	0	0	7
Springfield, Mass	0	0	------	0	2	0	5	0	53	0	0	4
Superior, Wis	0	0	------	0	0	0	0	0	3	0	0	6
Syracuse, N. Y	0	0	------	1	0	1	2	0	1	0	0	42
Tacoma, Wash	2	0	------	0	68	0	1	0	3	0	0	0
Tampa, Fla	0	0	------	0	0	0	1	0	0	0	1	0
Terre Haute, Ind	0	0	------	0	0	0	4	0	2	0	0	0
Topeka, Kans	0	0	------	0	1	0	0	0	7	0	0	0
Trenton, N. J	0	0	------	0	1	0	1	0	1	0	0	1
Washington, D. C	1	0	2	1	1	0	16	0	13	0	0	19
Wheeling, W. Va	0	0	------	0	1	0	1	0	2	0	0	15
Wichita, Kans	0	0	------	0	1	0	4	0	9	0	1	4
Wilmington, Del	0	0	------	0	0	1	1	0	0	0	0	3
Winston-Salem, N. C	0	0	------	0	0	0	2	0	1	0	0	5
Worcester, Mass	0	0	------	0	0	0	5	0	6	0	0	23

Anthrax—Cases: Philadelphia, 1.
Dysentery, amebic—Cases: New York, 1.
Dysentery, bacillary—Cases: Detroit, 3, Los Angeles, 6, New York, 5, Philadelphia, 1, Richmond, 2, San Francisco, 2.
Leprosy—New York, 1.
Tularemia—Cases: Detroit, 1, Pittsburgh, 1.
Typhus fever—Cases: Charleston, S. C., 1, Houston, 1, Nashville, 1, New Orleans, 1, New York, 1, Savannah, 2.

Rates (annual basis) per 100,000 population for the group of 87 cities in the preceding table (estimated population, 1942, 33,742,014)

Period	Diphtheria cases	Influenza		Measles cases	Pneumonia deaths	Scarlet fever cases	Smallpox cases	Typhoid and paratyphoid fever cases	Whooping cough cases
		Cases	Deaths						
Week ended Nov. 21, 1942	12.52	15.61	4.79	118.99	60.74	133.21	0.15	2.16	173.39
Average, 1937–41	19.05	16.08	[1] 3.57	[2] 142.24	[1] 53.94	120.38	0.78	4.53	171.91

[1] 3-year average, 1939–41.
[2] 5-year median.

PLAGUE INFECTION IN TACOMA, WASHINGTON

Under date of December 5, 1942, plague infection was reported proved in two flea specimens and in tissue of a rat taken in Tacoma, Wash.[1]

PSITTACOSIS

Reports of cases of psittacosis have been received as follows: Maryland, week ended August 22, 1942, 1; New York City, September 28, 1; Pennsylvania, week ended October 17, 4; Minnesota, week ended November 21, 1.

[1] For previous reports of plague infection found in pools of fleas and lice in Tacoma, see Public Health Reports, October 30, 1942, p. 1670, and November 13, 1942, p. 1742.

FOREIGN REPORTS

CANADA

Provinces—Communicable diseases—Week ended November 7, 1942.—During the week ended November 7, 1942, cases of certain communicable diseases were reported by the Dominion Bureau of Statistics of Canada as follows:

Disease	Prince Edward Island	Nova Scotia	New Brunswick	Quebec	Ontario	Manitoba	Saskatchewan	Alberta	British Columbia	Total
Cerebrospinal meningitis		2		2	4					8
Chickenpox		13	10	147	231	55	72	21	102	651
Diphtheria		13	1	32	2	8	2	1	2	61
Dysentery							1			1
German measles		2		22	7		5	1	6	43
Influenza		2				8			43	53
Measles		1		23	91	1	128		13	257
Mumps	2	25		118	311	1	26	50	108	641
Pneumonia					17	1	2		12	32
Poliomyelitis		2		3		3		1	1	10
Scarlet fever		6	17	90	100	26	18	24	40	321
Trachoma									2	2
Tuberculosis	3	7	12	95	37	23	15	11	25	228
Typhoid and paratyphoid fever			1	30	1	1	1	1		35
Undulant fever									1	1
Whooping cough		23		216	95	12	15	40	38	439
Other communicable diseases		2		7	274	46		2	4	335

COSTA RICA

Communicable diseases—August 1942.—During the month of August 1942, certain communicable diseases were reported in Costa Rica as follows:

Disease	Cases	Deaths	Disease	Cases	Deaths
Diphtheria	14	1	Typhoid fever	23	1
Measles	36		Whooping cough	8	
Poliomyelitis	3				

REPORTS OF CHOLERA, PLAGUE, SMALLPOX, TYPHUS FEVER, AND YELLOW FEVER RECEIVED DURING THE CURRENT WEEK

NOTE.—Except in cases of unusual prevalence, only those places are included which had not previously reported any of the above-mentioned diseases, except yellow fever, during the current year. All reports of yellow fever are published currently.

A cumulative table showing the reported prevalence of these diseases for the year to date is published in the PUBLIC HEALTH REPORTS for the last Friday in each month.

(Few reports are available from the invaded countries of Europe and other nations in war zones.)

Plague

Argentina—Cordoba Province.—Plague was reported in Cordoba Province, Argentina, as follows: March 1–31, 9 cases, 4 deaths; April 1–30, 3 cases; May 1–31, 3 cases; July 1–31, 1 case; September 1–30, 2 cases; October 1–31, 1 fatal case.

Indochina.—For the period November 1–10, 1942, 1 case of plague was reported in Indochina.

Typhus Fever

Egypt—Port Said.—For the week ended November 7, 1942, 3 cases of typhus fever were reported in Port Said, Egypt.

Rumania.—For the period November 1–10, 1942, 41 cases of typhus fever were reported in Rumania.

Turkey.—For the period November 1–10, 1942, 6 cases of typhus fever were reported in Turkey.

Yellow Fever

Togo.—During the week ended October 10, 1942, 1 case of yellow fever was reported in Togo.

COURT DECISION ON PUBLIC HEALTH

Milk—ordinance provisions upheld.—(California Supreme Court; *Natural Milk Producers Ass'n et al.* v. *City and County of San Francisco et al.*, 124 P.2d 25; decided April 2, 1942, as modified April 21, 1942.) A comprehensive milk ordinance of the city and county of San Francisco provided that market milk for sale and distribution for human consumption should consist of (a) certified milk, (b) guaranteed pasteurized milk, (c) grade A pasteurized milk, and (d) grade B pasteurized milk, and that no other milk should be sold for human consumption within the city and county. In effect the ordinance prohibited the sale of milk unless it was pasteurized, with the single exception of certified milk. In a suit to enjoin the enforcement of certain provisions of the ordinance, the plaintiffs, who were interested in the sale of guaranteed raw milk being permitted in San Francisco, first contended that the ordinance was invalid because it conflicted with the State law as embodied in the agricultural code. They asserted that that code permitted the sale of five grades of market milk, namely, certified, guaranteed raw, guaranteed pasteurized, grade

A raw, and grade A pasteurized, and that the ordinance prohibited the sale of guaranteed raw milk and grade A raw milk. The plaintiffs' view was that the agricultural code so completely occupied the field of milk regulation that there was no room for the operation of a municipal ordinance on the subject, but the Supreme Court of California said that it had long been the established general rule, in determining whether a conflict existed between a general and local law, that where the legislature had assumed to regulate a given course of conduct by prohibitory enactments a municipal corporation with subordinate power to act in the matter could make such additional regulations in aid and furtherance of the purpose of the general law as might seem appropriate to the necessities of the particular locality and as were not in themselves unreasonable. Mention was also made of section 451 of the agricultural code which provided in part: "No provision of this division, except subdivision (b) of section 458.1, or any rule and regulation of the director is a limitation on the power of a municipality or county to provide for reasonable additional regulations not in conflict therewith requiring standards higher than the minimum requirements for the grades of market milk established in this division." In answer to the plaintiffs' assertion that section 451 left to municipalities only the field of imposing stricter requirements upon the various grades of milk as established by the agricultural code and did not permit the complete prohibition of the sale of any one of the grades, the supreme court said that it was doubtful that there were more than three grades of milk specified in the said code, namely, certified, guaranteed, and grade A. "The latter two grades might be said to be divided into two kinds, raw and pasteurized." The court's view was that the ordinance merely imposed the additional restriction that the milk, whether it be guaranteed or grade A, had to be pasteurized and that, essentially, the requirement in the ordinance of pasteurization for both guaranteed and grade A milk was merely a higher standard for the grades which was not in conflict with the State law.

Another contention of the plaintiffs was that the ordinance was unconstitutional because discriminatory. They asserted that there was no substantial difference between guaranteed raw milk as defined in the agricultural code and certified milk as defined in the ordinance and that, therefore, there was no reasonable basis for forbidding the sale of one and not the other. The court, however, found itself unable to say that the ordinance was invalid on the ground stated, saying that a comparison of the two standards revealed without doubt that there was a substantial and reasonable difference which was directly related to the public health. The standards for guaranteed raw milk were reviewed and then the court proceeded to say that the standards for certified milk were quite similar but that, in addition, it was required

in the ordinance that certified milk conform "to the rules, regulations, methods, and standards for the production * * * of certified milk adopted by the American Association of Medical Milk Commissions and must bear the certificate of the milk commission of the San Francisco County Medical Society." It would seem, said the court, that, whatever may be the rules and regulations of the association, certified milk may be said to be subjected to a more rigid inspection than guaranteed raw milk by persons exceptionally well qualified therefor, that is, physicians and surgeons. "Furthermore, the agricultural code recognizes certified milk as being in a class by itself."

In their attack upon the ordinance, the plaintiffs also invoked the due process clause but, according to the court, it could not be said that the city and county had no reasonable grounds for requiring all milk sold therein to be either pasteurized or certified. It was true that the sale of milk was a lawful business protected by the Federal and State constitutions, but it could not be doubted that, as milk was vital to the welfare of the nation and susceptible of being a carrier of disease, the production, distribution, and sale thereof could be strictly regulated under the police power to safeguard the public health. The requirement that all milk be pasteurized was a proper police regulation and the fact that an exception was here made with reference to certified milk did not alter the situation. "Certified milk has long been established as milk in which especial precautions are taken to insure absence of disease and contamination, and the supervision is by especially qualified experts."

Another point urged by the plaintiffs was that there was an unconstitutional delegation of legislative power in that portion of the ordinance which provided that certified milk was market milk which conformed to the rules, regulations, methods, and standards adopted by the American Association of Medical Milk Commissions and had to bear the certificate of the milk commission of the San Francisco County Medical Society. The named association was said by the court to be a corporation, with its principal place of business in New York, whose members were the various local milk commissions throughout the nation. The San Francisco County Milk Commission consisted of five members appointed by the county medical society and a fee was charged those desiring to produce and sell certified milk to cover the costs of inspection and to purchase from the association the caps to be used on the containers. Legally the commission was not an administrative agency of either San Francisco or the State. But the court held that there was no unlawful delegation of legislative power, saying that, in conformity with the rule that when it may reasonably be done a statute should be interpreted in a manner that would avoid its being declared unconstitutional, it believed that "the requirement in the ordinance may be said to merely require that certi-

fied milk must meet standards established by a private corporation or group who are experts in the field, and that the legislative body was aware of those regulations and standards and by the ordinance merely made them a part thereof."

The judgment in favor of the defendants was affirmed.

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