in 1 mm^3 and the juvenile neutrophils (stabcells) from 99 to 1885 within 2 hr. Table I shows, furthermore, that there was first a short leucopenic stage as in most of the hitherto known forms of induced leucocytosis.[7]

In some instances (No. 170, 182) there was no primary leucocytic response after the injection of the kaolin and marked changes in the number of leucocytes and the blood picture did not appear before the 5th or 6th day.

Whether the mechanism of the primary and secondary leucocytosis is different remains questionable. Lindauer and Griffiths (*l.c.*) have found a pleocytosis of short duration in the cerebrospinal fluid in rats after injection of kaolin; however, it seems unlikely that the primary peripheral leucocytosis of the first days is due to an aseptic inflammatory irritation of the meninges as there were no signs of any meningeal irritation. For our problem the leucocytosis after the first days is more significant.

Summary. Intracisternal or intraventricular injection of a suspension of kaolin produces in rabbits a marked peripheral neutrophilic leucocytosis. These findings add another form of cerebrally induced leucocytosis to the other previously described; they furnish further support to the view of a central vegetative regulation of the cellular composition of the blood.

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Sulfanilamide, Sulfapyridine, Sulfathiazole and Sulfamethylthiazol in Experimental Gas Gangrene in Guinea Pigs.*

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(Introduced by E. W. A. Ochsner.)

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Since the publication of Domagk,[1] presenting the results of his experiments with Prontosil-S in streptococcal infections, numerous

[7] Doan, C. A., Zerfas, L. G., Warren, S., and Ames, O., *J. Exp. Med.*, 1928, **47**, 403.

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[1] Domagk, G., *Deut. med. Wchnschr.*, 1935, **61**, 250.

other investigators[2-6] have not only confirmed his work but also extended the scope of the usefulness of the derivatives of this drug.

The position which the drugs have in anærobic infections, especially that of gas gangrene, has not been clearly established. Bliss *et al.*,[5] and others[7, 8] described the effective action of sulfanilamide and its derivatives in experimental animals which had been injected with *Clostridium welchii.* Bohlman[9] reported 5 cases of gas gangrene treated successfully with sulfanilamide. Other authors[10, 11] have advocated the use of this drug in Clostridium infections. Unfortunately, the enthusiasm developing from these early reports was based largely on animal and *in vitro* experiments, which were not analogous to human infections. Recently, however, reports[12, 13] have appeared in the literature of the successful use of sulfanilamide in gas gangrene resulting from war injuries.

Clinical infections of the peritoneum due to the Clostridium group are rare, especially when consideration is taken of the frequency with which there is contamination due to the escape of intestinal contents following the traumatic or infectious break of the intestinal tract. Apparently, the peritoneum is able to resist contamination with *Clostridium welchii* more effectively than muscle tissue. The sacchrolytic nature of these organisms is well known and may partially account for this tissue susceptibility. Using sulfanilamide and sulfapyridine, Stephenson and Ross[14] were able to protect mice against several lethal doses of *Clostridium welchii* injected intraperitoneally. However, these same drugs proved ineffectual against a single lethal dose of the organisms intramuscularly.

A strain of *Clostridium welchii* which had been isolated from a fulminating case of gas gangrene obtained from Dr. Meleney's laboratory was used. Preliminary experiments showed this or-

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3 Long, P. H., Bliss, E. A., and Feinstone, W. H., *Pennsylvania Med. J.*, 1938-1939, **42**, 483.

4 Long, P. H., and Bliss, E. A., *Canad. Med. Assn. J.*, 1937, **37**, 457.

5 Bliss, E. A., Feinstone, W. H., Garrett, A. W., and Long, P. H., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 619.

6 Lockwood, J. S., *Penn. Med. J.*, 1938-1939, **42**, 1444.

7 Kendrick, Douglas B., *J. Clin. Invest.*, 1939, **18**, 593.

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9 Bohlman, H. R., *J. A. M. A.*, 1937, **109**, 254.

10 Kennedy, W. C., *Ill. Med. J.*, 1938, **73**, 260.

11 Sadusk, J. F., Jr., and Manahan, C. P., *J. A. M. A.*, 1939, **113**, 14.

12 Fuller, A. T., and James, G. V., *Lancet*, 1940, **1**, 487.

13 Jocelyn Swan, R. H., *Brit. Med. J.*, 1940, **2**, 97.

14 Stephenson, D., and Ross, H. E., *Brit. Med. J.*, 1940, **1**, 471.

ganism to be particularly virulent for guinea pigs. However, during the course of the experiments, this high degree of virulence diminished but was reestablished partially by serial animal passage.

This strain was found to be particularly virulent for guinea pigs when injected into the thigh muscles especially when followed by pressure, gently traumatizing the area injected. The organisms were grown in dextrose beef heart broth for varying lengths of time of 6 to 18 hr. In a few instances, the cultures were centrifugalized, the organisms separated, washed and resuspended in the proper dilutions of saline or supernatant broth. Solutions of sulfanilamide, sulfapyridine, sulfathiazole, and sulfamethylthiazol were injected intraperitoneally in 50 mg and 100 mg doses, the sodium salts of the last 3 drugs being used. Each animal received 3 doses of medication during the 24 hr period prior to the inoculation with organisms, and additional doses 3 times daily at 4 hr intervals during the day for varying lengths of time. By the injection of medication previous to inoculation with organisms, it was hoped to establish sufficient circulating medication before the development of an overwhelming

TABLE I.

Dose No. bacteria injected	Medication* Dosage in mg		No. guinea pigs	No. surviving at hours 18	26	42	48	66	72	96	120	Survived No.	Day
2200×10^6	San	50	16	8	7	4	2	0	—	—	—		
		100	6	5	4	0	—	—	—	—	—		
	Sp	50	16	10	8	2	2	0	—	—	—		
		100	6	3	2	0	—	—	—	—	—		
	St	50	16	16	13	4	3	2	2	0	—		
		100	6	6	4	0	—	—	—	—	—		
	Smt	50	16	14	11	5	4	1	1	1	0		
		100	6	5	1	0	—	—	—	—	—		
	Control		10	3	2	1	0	—	—	—	—		
			4	1	0	—	—	—	—	—	—		
1200×10^6	San	50	16	9	7	4	4	2	2	2	2	1	7
												0	8
	Sp	50	16	11	9	4	3	2	2	0	—		
	St	50	16	16	15	12	12	9	8	8	5	4	6
												2	7
												1	9
												0	15
	Smt	50	16	14	11	5	3	2	2	2	0		
	Control		10	5	2	0	—	—	—	—	—		
0	San		4	3 survived; 1 died on 5th day									
	Sp		4	"									
	St		4	"									
	Smt		4	2 " 1 died on 3rd day and 2 on 5th day									

*Smt = Sodium salt of sulfamethylthiazol.
St = Sodium salt of sulfathiazole.
Sp = Sodium salt of sulfapyridine.
San = Sulfanilamide.

infection. Blood levels of the various drugs were not determined in any instance.

The guinea pigs inoculated with 0.4 cc of a dextrose beef heart broth culture died within 16 hours with a typical picture of gas gangrene. Because of the unusually high virulence, studies of progressively decreasing numbers of organisms obtained from 6 hour cultures, washed and resuspended in saline, showed that it was possible to kill guinea pigs with 0.12 cc of 320×10^6 organisms per cc, or a total of 40 million organisms. Using this smaller number, the survival time was increased to approximately 24 hours.

Experiment I. This experiment was performed in an attempt to determine the minimal number of *Clostridium welchii*, which when injected intramuscularly into guinea pigs receiving medication, would produce a fatal gas gangrene. It was found that the medications did not protect guinea pigs inoculated with 50×10^6 organisms, washed and resuspended in saline. Fewer organisms did not consistently produce death in either the medicated or control animals. None of those receiving sulfanilamide survived more than 48 hours. Sulfapyridine indicated an increased protective value in that it was able to protect all but one of the guinea pigs for 7 days or more; one, inoculated with 200×10^6 organisms, died within 42 hours. Of the 8 guinea pigs receiving sulfamethylthiazol, 3 died within 72 hours, 3 of the 5 remaining died on the 5th, 7th, and 8th days respectively, and 2 survived the infection. Sulfathiazole seemed to offer slightly more protection than the other three medications; of the 8 guinea pigs receiving this drug, 2 died on the 3rd day, one each on the 5th and 6th days respectively, and 4 survived the infection. Medication and organism controls were run throughout the experiment.

Experiment II. As the virulence of the strain had decreased, restoration was attempted by repeated animal passage. Since the virulence could not be brought back to its original level, it was necessary to increase the size of the inoculum to contain a minimum of 1200×10^6 organisms. Sixteen guinea pigs were used for each medication and each quantity of inoculum, with suitable controls. Of the 32 guinea pigs receiving sulfanilamide, 26 died within 48 hours, 4 within 66 hours, and the remaining two survived until the 7th and 8th days. Similar results were found in the sulfapyridine group, as all died within 4 days. Of the 16 animals receiving 2200×10^6 organisms and being treated with sulfathiazole, 13 died within 48 hours, one during the third day, and the remaining 2 died on the 4th day. Of the 16 guinea pigs receiving sulfathiazole and the smaller inoculum of 1200×10^6 organisms, 8 survived 3 days;

of these, 3 died on the 5th day, one on the 6th day, 2 on the 7th day, and 2 survived the infection but died of other causes on the 9th and 15th days. Of the group receiving sulfamethylthiazol, all 32 guinea pigs succumbed by the 5th day; however, the death rate was not quite as rapid as of those receiving sulfanilamide and sulfapyridine. Of the 20 organism controls, all but 4 died within 24 hours, and of the remaining 4, three died within 42 hours and one within 48 hours.

Due to the possibility that the dose of the medications employed in the preceding experiments was inadequate to demonstrate protection for the guinea pigs infected with *Clostridium welchii,* in another group of guinea pigs the dose of medication was increased to 100 mg, or twice that previously used. This was the only significant change in this experiment. However, even with this larger dose of medication, the results were similar to those observed in the experiments in which 50 mg of medication were used. All the animals died with gas gangrene within a period of 41 hours irrespective of medication. All organism controls were dead within 22 hours. Of the medication controls, the 2 receiving sulfamethylthiazol were dead on the 5th day, and the others were sacrificed on the same day for tissue study. From this experiment it can be concluded that the 100 mg doses of drug were no more effective than the 50 mg doses in protecting these guinea pigs.

In the experiments reported, autopsies were done on all of the animals which succumbed to the infection. Those receiving sulfanilamide and sulfapyridine developed a more extensive infection involving the musculature of the abdominal wall and spreading to the opposite side. The changes observed in the muscles appeared to be more profound; in many instances, the thigh muscles had undergone complete degeneration with lysis. In those pigs treated with sulfamethylthiazol and sulfathiazole, the lesion was different, in that the pathological changes were limited to the immediate adjacent muscle groups. Even though the lesion had spread to the abdomen in a few of these guinea pigs, in the majority of those receiving sulfathiazole and sulfamethylthiazol the involvement was not as extensive as those receiving sulfanilamide and sulfapyridine. *Clostridium welchii* was recovered from all lesions at autopsy.

From these experiments, it is impossible to conclude that these drugs are protective in *Clostridium welchii* infections of the thigh muscles of guinea pigs. The protection offered by these medications is very slight and varies somewhat with the medication used. Apparently, sulfanilamide and sulfapyridine intraperitoneally were of little benefit and in no way modified the course of the infection.

Even though most of the pigs treated with sulfathiazole and sulfamethylthiazol developed typical gas gangrene and succumbed, the development of the infection was delayed and in many instances was not as extensive as those seen in the guinea pigs treated with sulfanilamide and sulfapyridine. From this, the conclusion may be drawn that sulfamethylthiazol and sulfathiazole had slight therapeutic advantage over sulfanilamide and sulfapyridine in *Clostridium welchii* infections in guinea pigs.

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Further Experiments with Deplanted and Deranged Nerve Centers in Amphibians.*

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A previous communication[1] has described the appearance of rhythmic spontaneous (=endogenous) activity, and later of reflexes, in fragments of spinal cord transplanted ("deplanted") to the dorsal fin of amphibian larvæ and provided with grafted limbs as effectors. A continuation of these studies has brought the following results.

A large section of *spinal cord* was deplanted to the fin and made to innervate 2 limbs, one grafted anteriorly and the other posteriorly. After re-innervation, prolonged seizures of spontaneous activity appeared in both limb grafts. During all major fits the beats of the 2 limbs occurred synchronously and with approximately proportional strength. This proves that in all actions, except very weak ones, the whole neurone pool of the grafted center discharges in unison. Reflexes likewise spread through the whole grafted center without localization.

Some limbs were deplanted along with their own spinal ganglia and cord segments, with all peripheral nerve connections being left intact. During the days following the operation this isolated reflex preparation gave orderly reflex responses. Gradually, however, the response deteriorated, assuming undifferentiated mass character with prolonged after-discharges, while at the same time spontaneous rhythmic

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1 Weiss, P., PROC. SOC. EXP. BIOL. AND MED., 1940, 44, 350.