which seemed to be coincident with mechanical relaxation or with the reflected wave seen in the slow-motion pictures of contracting cultures.[4] Close examination of the records would seem to suggest that such after-potentials occur with each beat in the more quickly beating cultures (150 per min.) but are incorporated in the record of the following beat. Faster galvanometer and film speeds would be necessary to demonstrate this.

Efforts to obtain diphasic responses were unsuccessful when the electrodes were placed one to 2 cell lengths apart along a 2 to 3 cell strand of contracting muscle. The only diphasic record was obtained by putting one electrode near each of 2 negative centers which were slightly out of phase.

With the unaided eye it is impossible to say whether the electrical centers serve also as centers for the spread of mechanical contraction or not. No apparent difference was found in the action potentials of non-striated cultures and those in which striation was almost complete.

An attempt was made to register the potential changes across the membrane of a single contracting cell, but failed because the excessive vibration of the inserted electrode caused almost immediate death of the cell as judged by refractive properties. The rather characteristic "death wave", Fig. 1c, shows a sudden large negative rise (which may be 20 millivolts) followed by an almost equally rapid fall to about half this value or less. Small variations on some of these records were attributed to contractions of neighboring cells.

7656 P

Note on the Metabolism of Copper in Splenectomized Rabbits.

MARTA SANDBERG AND OLIVE M. HOLLY.

From the Laboratory Division, Montefiore Hospital, New York City.

In previous studies[1] it was found that removal of the spleen in albino rats free from *Bartonella muris* infection is followed by an increased elimination of copper, which produces a negative copper balance. In a study of the metabolism of rabbits before and after splenectomy we found that removal of the spleen causes an increase in copper excretion in this species as well.

[4] Goss, C. M., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 292.

[5] Goss, C. M., *Arch. f. Exp. Zellforsch.*, 1933, **14**, 175.

[1] Sandberg, M., and Perla, D., *J. Exp. Med.*, 1934, **60**, 395.

Two rabbits were splenectomized at the age of 2½ months, after a foreperiod of 3 weeks, while another one was kept as a control under identical conditions. They were given alfalfa hay, oats and copper-free water, which was also used for all chemical determinations and for washing everything that came in contact with the animals. Urine was collected daily, feces twice a week. The urine was analyzed for total nitrogen (Kjeldahl), total sulphur (Benedict), total and inorganic sulphates (Folin), calcium (volumetric), phosphorus (Fiske-Subbarow), and copper.[2] Feces, hay and oats were analyzed for total nitrogen, calcium, phosphorus, copper and iron.[3]

Our experiments show that splenectomy causes no change in the metabolism of nitrogen, sulphur, phosphorus and iron in rabbits. The calcium excretion proceeds unchanged after splenectomy, which also tends to show that the spleen is not involved in the regulation of calcium metabolism.

While the excretion of copper in the intact animal was remarkably constant, it increased in the splenectomized animals a week after the operation. The increase takes place in the feces, since the greater part of copper is excreted by the gut. The excretion of copper by the kidney does not seem to be influenced by splenectomy, though a larger amount of the total copper excreted is found in the urine in rabbits than in rats. As shown in the table, the copper intake on a diet of hay and oats is insufficient to maintain a positive copper balance in a growing rabbit, but the loss of copper from the body

TABLE I.
Daily Average of Copper Excretion per Week.

	Splenectomized Rabbit					Normal Rabbit				
	Urine	Feces	Total Excretion	Intake	Retention	Urine	Feces	Total Excretion	Intake	Retention
1934	mg.	.mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
May 7-13	.156	.437	.593	.536	—.057	.202	.456	.658	.538	—.120
14-20	.156	.446	.602	.548	—.054	.218	.443	.661	.543	—.118
21-27	.164	.438	.602	.543	—.059	.223	.450	.673	.544	—.129
28-*										
June 3	.168	.453	.621	.557	—.064	.232	.455	.687	.557	—.130
4-10	.164	.545	.709	.559	—.150	.226	.451	.677	.558	—.119
11-17	.113	.868	.982	.544	—.438	.230	.452	.682	.549	—.133
18-24	.114	.653	.767	.540	—.227	.220	.453	.673	.546	—.127
25-										
July 1	.155	.794	.949	.545	—.404	.214	.448	.662	.546	—.117
2-8	.144	.573	.717	.538	—.179	.224	.446	.670	.549	—.121
9-15	.146	.565	.711	.542	—.169	.232	.450	.683	.553	—.130

*Splenectomized May 28th.

[2] McFarlane, W. D., *Biochem. J.*, 1932, **26**, 1022.

[3] Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1926, **67**, 43.

increases considerably after splenectomy. This further supports the evidence presented in our study of the copper metabolism of rats regarding the rôle of the spleen in the utilization of copper.

7657 P

Shwartzman Phenomenon with B. Pertussis Culture Filtrates.

LEWIS HENRY KOPLIK. (Introduced by Gregory Shwartzman.)

From the Laboratories of the Mount Sinai Hospital, New York.

Attempts to reproduce the Shwartzman phenomenon with *B. pertussis* have been reported by several authors. (Gross,[1] Shwartzman,[2] Mishulow, Mowry and Scott.[3]) However, the potency of the filtrates seemed to have varied for unknown reasons and always remained low as compared to similar preparations from *B. typhosus,*[4] meningococcus[5] and other organisms.[6]

During some work with serum neutralizations of *B. pertussis* toxic substances it occurred to the author of this paper to employ Toomey and McClelland's brain veal infusion medium[7] for the preparation of the toxic factors necessary for the production of the Shwartzman phenomenon.

In these experiments the brain medium was prepared essentially according to the method of Toomey and McClelland with various peptones (neopeptone, proteose-peptone (Difco), Witte's peptone). The H-ion concentration was adjusted either to pH 7.3 or 7.8. The media were seeded with 24-hour-old cultures on "chocolate agar" slants. The strain used was subcultured for at least 3 successive days preceding these inoculations.

The organisms grew luxuriantly in the brain media similar to Toomey and McClelland's description. The strain employed was M 12 (group B), kindly supplied to us by Miss Mishulow of the New York City Board of Health Laboratories.

---

1 Gross, Louis, personal communication.

2 Shwartzman, Gregory, *J. Exp. Med.*, 1930, **51**, 581.

3 Mishulow, Lucy, Mowry, Isabelle W., and Scott, Eleanor B., *J. Immunol.*, 1930, **19**, 227.

4 Shwartzman, Gregory, PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 843.

5 Shwartzman, Gregory, *J. Inf. Dis.*, 1929, **45**, 232.

6 Shwartzman, Gregory, PROC. SOC. EXP. BIOL. AND MED., 1928, **26**, 207.

7 Toomey, John A., McClelland, Joseph E., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 34.