gestion even to the point of capillary hemorrhage, especially in the mucosa of the stomach and duodenum. The spleen less regularly presented the classical picture of constriction and pallor, due we believe to the acute nature of the experiments. One animal injected subcutaneously (0.37 ml) showed almost complete hemolysis. The 3 control animals with ether anesthesia alone showed an average hemoconcentration of less than 5%. Pathologically, their tissues were normal.

Summary and Conclusions. Acrolein injected into experimental animals by any of several routes produces a condition identical in its clinical and pathological manifestations to that generally accepted as "shock." By using the intravenous route and relatively large doses in certain animals, it is believed that this picture is the direct result of capillary damage by acrolein or lipoid breakdown products, rather than the result of the liberation of "H" substance or other tissue breakdown protein substances. Studies concerning the development of antibodies to acrolein in the serum of burned animals and individuals are in progress.

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Action of Bacterial Toxins on Tumors. IV. Distribution of Tumor-Hemorrhage Agents Among Bacterial Species.*

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In a survey[1] ranging over 28 genera and nearly 100 strains of bacteria it was found that bacterial substances capable of inducing hemorrhage in implanted tumors were restricted to gram-negative species. A close correlation between the occurrence of hemorrhage-producing agents and the toxic somatic O antigens of gram-negative bacteria was adduced. Further investigations demonstrated a close parallelism between the lethal and the tumor-hemorrhage properties of purified bacterial products[2] and a parallel inhibition of both of these actions by sulfonamide drugs.[3]

Since the O antigens of gram-negative bacteria (and the chemically similar Vi antigens) have been identified in several recent studies with the essential immunizing constituents of antibacterial vaccines, it was considered of interest to explore further, in both pathogenic and non-pathogenic bacteria, the distribution of toxins inducing the type of vascular damage which we believe to be characteristic of the O and Vi antigens. The present study is an extension of the original survey, and includes the testing for tumor-hemorrhage activity of an additional 30 gram-negative and 7 gram-positive species.

Methods. The hemorrhage activity of concentrated bacterial cultures was tested on white male Rockland mice bearing 7-day-old implants of Mouse Sarcoma 180. In preparing mice for assay, a 2-3 mm cube of tumor tissue was implanted subcutaneously near the axilla and allowed to grow for 7 days, by which time it had become well vascularized and had developed into a spherical mass about 1 cm in diameter. The material to be assayed was administered intraperitoneally. The bacteria were killed by addition of a small quantity of toluene-chloroform mixture. This treatment is unlikely to inactivate the O antigen.[4] Where necessary the volume was reduced by pervap-

* We wish to express our gratitude to Prof. C. B. Van Niel, who supplied most of the cultures used in this survey.

1 Zahl, Paul A., Hutner, S. H., Spitz, S., Sugiura, K., and Cooper, F. S., *Am. J. Hyg.*, 1942, **36**, 224.

2 Zahl, Paul A., and Hutner, S. H., PROC. SOC. EXP. BIOL. AND MED., 1943, **52**, 116.

3 Zahl, Paul A., and Hutner, S. H., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 285.

4 Ungar, J., Jenner, R. M., and Hunwicke, R. F., *J. Path. and Bact.*, 1942, **54**, 331.

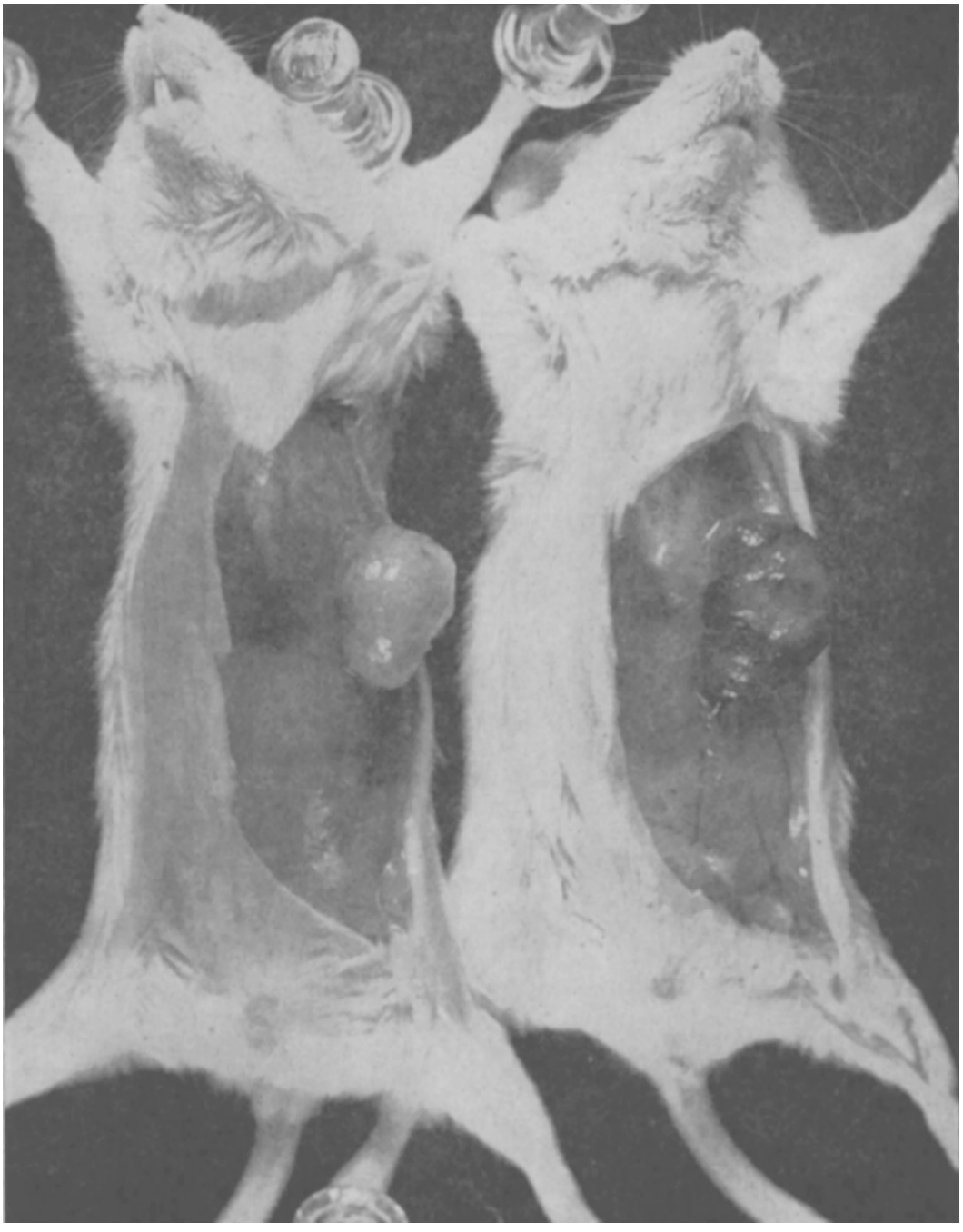

FIG. 1.

Comparison of Normal and Hemorrhagic Tumors.

Left: Mouse with normal seven-day-old Sarcoma 180.

Right: Mouse with hemorrhagic seven-day-old Sarcoma 180. Hemorrhage was induced by the intraperitoneal injection of dead *Salmonella typhosa* suspension about six hours previous to dissection.

oration through cellulose tubing.† An effort was made to inject doses approaching the lethal limit while keeping the volume injected to less than 0.5 ml. Hemorrhage in tumors reaches its maximum within 8 hours after injection of the toxin. The animals at this stage were sacrificed, and the tumors dissected. The degree of hemorrhage was graded as slight (*), considerable (**), and marked (***). Normal tumor tissue is pink; a hemorrhagic tumor is deep red to deep purple (Fig. 1).

Table I lists the bacterial species thus tested. For comparative purposes, the bacterial genera tested in the original survey are listed below.‡

Discussion. If these toxins, which are generally associated with pathogenicity, are also to be found in non-pathogenic species, then it becomes increasingly probable that the hemorrhage-inducing substance is characteristic of a larger class of organisms, namely, the gram-negative bacteria. Therefore, in the present extension of the initial survey, special attention was paid to a number of non-pathogens and in particular to photosynthetic bacteria and to the *Pseudomonas* complex of species considered by Stanier and Van Niel[5] to represent a relatively unspecialized group.

An examination of Table I will disclose an interesting example of the toxicity of a presumably unspecialized and non-pathogenic bacterium, *Rhodospirillum rubrum.* This photosynthetic gram-negative purple bacterium gave marked tumor-hemorrhage.§ This, coupled with the widespread occurrence of tumor-hemorrhage agents in the *Pseudomonas* group, may be regarded as a strong indication that tumor-hemorrhage toxins are conservative evolutionary characters not necessarily associated with a pathogenic tendency; still, the possession of these toxins suggests that with the addition of invasiveness a harmless gram-negative bacterium may be converted into a pathogen.

It seems reasonable to suppose that tumor-hemorrhage toxins, whether found in pathogens or non-pathogens, are similar; and presumably identical with those ordinarily observed during infections by gram-negative bacteria.‖

Decision as to whether the hemorrhage factor is invariably bound up in a complex O antigen on the *Shigella-Salmonella* model would require separate investigation of each species.

Since it has been shown repeatedly that rough strains lack the O antigen, it is obvious that unless attention is paid to the possibility of smooth-rough transformation, misleading results are likely to be obtained with stock cultures of unstable species. This may explain why *Salmonella typhosa* strains tested in our initial survey did not induce hemorrhage, whereas the virulent Panama strain of *S. typhosa* tested in the present study was highly hemorrhagic. Similarly, *Pasteurella cuniculicida* elicited hemorrhage, whereas *P. boviseptica* and *P. cuniculicida* had been previously reported inactive.[6] Inasmuch as a comparative study of tumor-hemorrhage as a function of the presence of O antigens may supply an

† Purchased from the Sylvania Industrial Corporation, New York City.

‡

Gram-Negative	Gram-Positive
Aerobacter (hem.)	*Bacillus* (no hem.)
Brucella (hem.)	*Actinomyces* (no hem.)
Chromobacterium (hem.)	*Clostridium* (no hem.)
Escherichia (hem.)	*Corynebacterium* (no hem.)
Hemophilus (hem.)	*Gaffkya* (no hem.)
Klebsiella (hem.)	*Mycobacterium* (no hem.)
Neisseria (hem.)	*Pneumococcus* (no hem.)
Pasteurella (no hem.)	*Sarcina* (no hem.)
Proteus (hem.)	*Staphylococcus* (no hem.)
Pseudomonas (hem.)	*Streptococcus* (no hem.)
Salmonella (hem.)	
Serratia (hem.)	
Shigella (hem.)	
Vibrio (uncertain)	

§ According to Stanier and Van Niel[5] all photosynthetic bacteria are gram-negative. We have found this true of the photosynthetic purple bacteria *Streptococcus varians* and *Rhodobacillus palustris,* neither of which however induced tumor-hemorrhage. It should be noted that *Streptococcus varians* appears to be a rod rather than a streptococcus in the usual morphological sense, and that *Rhodobacillus palustris* is not a spore-former.

‖ The simultaneous presence of exotoxins, as in *Shigella dysenteriæ,* some strains of *E. coli,* etc., must of course be taken into consideration.

[5] Stanier, R. Y., and Van Niel, C. B., *J. Bact.,* 1941, **42**, 437.

[6] Gardner, R. E., Bailey, G. H., and Hyde, R. R., *Am. J. Hyg.,* 1939, **29** (Sec. B), 1.

TABLE I.

Species	Source of Cultures	Effect of bacterial suspensions on tumors Numerator = degree of hemorrhage Denominator = No. of animals tested		
Gram-Negative:				
Aerobacter aerogenes	Van Niel	***/3	**/1	
" *cloacæ*	" "	***/5		
Acetobacter sp.	" " strain 2.1	***/2	**/1	*/2
Alcaligenes (bacterium) radiobacter	" "	***/7	**/2	
Brucella abortus	H. L. Gilman, N. Y. State Veterinary Coll. Phenolized vaccine	***/5	**/5	
Hydrogenomonas sp.	Van Niel	***/3	**/1	
Neisseria catarrhalis	ATCC 7900	***/2	**/3	
Pasteurella cuniculicida	" 7277	***/3	**/2	
Phæobacterium sp.	Van Niel	***/6	**/2	0/1
Phæomonas varians	" "	*/1	0/7	
Protoaminobacter alboflavus b	" "	***/4	**/2	
Pseudomonas acidovorans	" "	***/6	**/1	*/1
Ps. caryocyaneus	" "	***/4		
Ps. droebachense (marine agar digester)	" "	***/3	**/1	
Ps. indoloxidans	" "	***/3	**/2	0/1
Ps. iridescens (marine agar digester)	" "	***/2	**/2	
Ps. ovalis	" "	***/5		
Ps. punctata	" "	***/5		
Ps. savastanoi	" "	***/3	**/2	
Ps. stutzeri	" "	***/3	**/1	*/1
Ps. sp. (marine nodule-former)	" "	***/3	**/1	*/1
Ps. (Hydrogenomonas) sp. (not identical with *Hydrogenomonas* above)	" "	***/2	**/2	
Rhizobium meliloti	ATCC 4401	0/5		
Rhodobacillus palustris (photosynthetic)	Van Niel	0/11		
Rhodospirillum rubrum (photosynthetic)	" "	***/7	**/6	*/1
Salmonella typhosa (Panama strain)	G. F. Luippold, U. S. Army Med. School	***/5	**/2	
Spirillum sp. (marine)	Van Niel	***/2	**/2	*/1
Streptococcus varians (photosynthetic)	" "	0/8		
Vibrio beijerincki (marine agar digester)	" "	***/2	**/2	
Vibrio tyrogenus	ATCC 7085	***/3	**/2	
Gram-Positive:				
Aerobacillus polymyxa	Van Niel	0/7		
Erysipelothrix muriseptica	ATCC 4162	0/6		
" *rhusiopathiæ*	" 805	0/5		
Listeria monocytogenes (1)	" 984	***/3	**/1	*/1
" " (2)	" 4428	***/1	**/1	0/1
" " (ovine) (3)	P. Olafson, N. Y. State Veterinary Coll.	0/5		
Micrococcus cyaneus	Van Niel	0/7		
Rhodococcus agilis	" "	0/7		
Sporosarcina ureæ	" "	0/6		
Non-Bacterial:				
Cytophaga krzemieniewskæ	" "	0/5		

ATCC = American Type Culture Collection.
*** = marked hemorrhage.
** = considerable hemorrhage.
* = slight hemorrhage.
0 = no hemorrhage.

independent test of the postulated relation between the O antigens and the tumor-hemorrhage agents, we propose to examine this point in detail later.

We are unable at the present time to account for the absence of a demonstrable toxin in *Rhizobium, Phœomonas, Streptococcus varians* and *Rhodobacillus palustris.*

The two isolates of *Listeria* are the only gram-positive organisms observed to yield hemorrhage-toxins. The taxonomic position of this species is uncertain, yet *Erysipelothrix,* to which *Listeria* may be closely related,[7,8] was inactive. The anomalous systematic position of *Listeria* has been recognized by Stanier and Van Niel.[5]

The lack of hemorrhage with *Cytophaga krzemieniewskœ* is not conclusive as only small amounts of cell body were available. A study of more material and other members of the *Myxobacteriales* is needed to determine whether the gram-negativity of this group is indicative of a relationship to gram-negative bacteria, if tumor-hemorrhage constitutes a criterion.

Conclusions. An extension was made of an earlier survey of bacterial substances inducing hemorrhage in implanted mouse tumors. It was found that with a few exceptions gram-negative forms induced tumor-hemorrhage, while this property, with one exception, was lacking in the gram-positives. An interesting example was the induction of hemorrhage by the photosynthetic gram-negative purple bacterium *Rhodospirillum rubrum.* It is concluded that toxins inducing tumor-hemorrhage, and probably bound up in the complex O antigens, are characteristic of gram-negative bacteria.

[7] Barber, Mary, *J. Path. and Bact.*, 1939, **48**, 11.

[8] Hutner, S. H., *J. Bact.*, 1942, **43**, 629.

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Lung Tumors Following Intraperitoneal Injection of 1:2:5:6-Dibenzanthracene into Young Mice of Three Strains.

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Data have already been published[1] demonstrating inherited differences in degree of susceptibility to spontaneous and to tar-induced lung tumors in two inbred strains of mice when the criteria for susceptibility included not only the percentage of tumor-bearing animals per group but also the number of tumor nodules per individual. The same criteria revealed constitutional differences in susceptibility among 3 strains of mice observed after intraperitoneal injections of 1:2:5:6-dibenzanthracene into very young animals.[2] The present paper reports further data obtained after intraperitoneal injections, combined with the original observations made following this technic.

The three strains used, the Swiss, our branch of the Bagg albinos, and No. 1194,* differ in susceptibility to spontaneous neoplasms. The Swiss have the highest lung tumor incidence. In a sample of 448 mice (Table I), about one-half, or 46.1%, had tumor. This figure is based upon mice of all ages. However, tumor incidence increases with age, and, in groups of mice of this strain that have reached a year and a half or more, 80% have tumor. Tumors occur early in this strain, a growth having been observed in a mouse only 3 months of age. One of the characteristics of lung tumor is that the nodules may be multiple. In the Swiss strain, of those animals that have tumor, almost half have multiple nodules, the largest number of nodules observed in a single

[1] Lynch, C. J., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 186.

[2] Lynch, C. J., *Cancer Research*, 1941, **1**, 740.

* Source of material: Swiss-8, (8117, 8123); Bagg-L (1916, 1917); Strain No. 1194, (421, 453).