same sample (Table VI). No correlation was observed. One of the two samples of blood showing the least bactericidal power, contained the highest concentration of complement and the 2 samples having the greatest killing power yielded very low complement titer.

Finally the thermostability of the active factor was tested by removing the serum as completely as possible from samples of defibrinated blood after centrifugation and heating it at 55°C. for 30 minutes. The inactivated serum was then restored to the cells from which it had been removed and bactericidal tests run. For controls we used a part of the same sample, subjected to the same treatment with the exception that the serum was not heated. In a few cases a part of the serum was heated to 68°, returned to the cells and then tested for killing effect. Similar tests were made with serum alone after heating at 55° or 68° for 30 minutes and with unheated controls. In none of these cases was there any evidence that the bactericidal property was impaired by the heating of the serum.

Conclusions. The power of defibrinated blood to inhibit the growth of or destroy streptococci is dependent upon the presence of surviving leucocytes and is roughly proportional to the number of leucocytes, other factors being equal. A minimal number of leucocytes is necessary for the demonstration of the bactericidal effect. The bacteria are removed by phagocytosis and finally destroyed by intracellular lysis. Complement is not necessary for the bactericidal action of defibrinated blood or serum. The bactericidal element in defibrinated blood which is effective against streptococci is not destroyed by heating the separated serum at 55°C. for 30 minutes. The results of a small number of tests suggest that the heating of the serum even at 68°C. does not impair the bactericidal effect of the restored defibrinated blood.

7543 P

Development of Gastric Ulcers and Decrease in Reducing Power of Adrenals Following Injection of Bile Salts.

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Sellards[1] reported the development of acute gastric ulcers in guinea pigs following intraperitoneal bile salt injection. His ob-

servations have been confirmed and extended by Tashiro and coworkers.[2] We injected approximately 40 pigs with varying amounts of bile salts (Fairchilds). The expected individual variations in susceptibility were observed, but injections of 0.1 gm. or more generally caused death and extensive ulceration of the gastric mucosa. The latter was usually so marked that the site of the ulcers could be plainly observed from the outer surface of the stomach, appearing as thin, semi-transparent areas. In 3 animals perforation occurred, gastric contents being found in the peritoreal cavity at autopsy.

Szent-Gyorgyi[3] reported the darkening of the adrenal cortex when subjected to a silver nitrate solution. Harris and Ray[4] and Siehrs and Miller[5] report that this does not occur in guinea pigs on a scorbutic diet. When we attempted to stain, with silver nitrate, the adrenals of pigs previously injected with bile salts, darkening was slight or absent. Adrenals of apparently normal pigs may, however, not stain with silver nitrate. Gough and Zilva[6] report that in pigs given 10 cc. of decitrated lemon juice daily for a period of 3 months the adrenals did not stain, although at autopsy no abnormalities were observed. We kept 12 pigs on a diet of oats, an occasional carrot, and 2 cc. of orange juice daily for one week. The cortex of the adrenals did not stain with silver nitrate. The absence of this staining reaction may merely indicate an insufficient excess of vitamin C in the diet to allow for its deposition in the adrenal. Therefore, in the remaining experiments, a diet abundant in vitamin C was administered, the pigs receiving oats, fresh carrots and cabbage, and, in addition, 4 cc. of orange juice daily for a period of 3 weeks prior to injection. The adrenals of control animals stained almost totally black with 0.4% silver nitrate solution when exposed for 3 minutes to a 115 watt blue mazda lamp at a distance of approximately 8 inches. The adrenals of animals injected with bile salts also showed some reduction under similar treatment, but in practically all cases the extent of darkening was distinctly less than in the controls and tended more toward a brown than to the black of the controls. The decrease in reducing power was also confirmed by iodine titration, which, although not specific for vitamin

[1] Sellards, A. W., *Arch. Int. Med.*, 1909, **4**, 502.

[2] Tashiro, *et al.*, *Med. Bull. Univ. Cincinnati*, 1931, **6**, 110, 124, 130, 134, 144.

[3] Szent-Gyorgyi, A., *Biochem. J.*, 1928, **22**, 1387.

[4] Harris, L. J., and Ray, S. N., *Biochem. J.*, 1933, **27**, 303.

[5] Siehrs, A. E., and Miller, C. O., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 696.

[6] Gough, J., and Zilva, S. S., *Biochem. J.*, 1933, **27**, 1279.

C, should reveal any marked decrease in this substance. Unfortunately at the time these experiments were conducted, we did not have available 2,6-dichlorophenolindophenol, which, according to recent work, exhibits considerable specificity for ascorbic acid.[7] The technique consisted in grinding the whole adrenal under 10% trichloracetic acid, filtering, and washing the residue, followed by titration of the filtrate with 0.002 N iodine, using starch as the indicator. For titration the left adrenal was generally employed, the right having been used for silver nitrate staining. From Table I it

TABLE I.
Iodine Titration of Adrenals Following Bile Salt Injection.

Sex	Wt. gm.	Dose bile salts	Time for death	Wt. adrenal gm.	cc. I_2	cc. I_2/gm. adrenal
M	234	control	killed	.065	.65	10.0
M	226	"	"	.065	.61	9.4
F	187	"	"	.034	.35	10.3
F	251	"	"	.074	.75	10.1
M	265	"	"	.042	.62	14.8
M	291	"	"	.047	.50	10.6
F	254	"	"	.053	.77	14.5
M	188	.05 g.	4¼ hrs.	.061	.40	6.6
M	167	.10	5¼	.073	.61	8.3
M	207	.10	2¼	.075	.16	2.1
M	200	.15	1¼	.059	.50	8.5
F	187	.10	4¼	.038	.40	10.5
F	175	.15	1¼	.050	.27	5.4
F	205	.15	2½	.067	.20	3.0
F	—	.15	2½	.076	.48	6.3
M	312	.05	26	.066	.53	8.0
M	286	.10	4	.064	.63	9.8
M	304	.15	2	.075	.57	7.6
F	304	.10	4	.052	.38	7.3
F	292	.10	4½	.082	.69	8.4
F	290	.15	2	.067	.46	6.9
M	332	.10 initial .05 23 hrs. .05 27 " .10 29 "	30½	.067	.56	8.3
F	294	.05 initial .05 23 hrs. .05 26 "	31	.102	.80	7.8

Average cc. iodine/gm. adrenal controls = 11.4
injected pigs = 7.2

will be observed that injected animals showed a decreased iodine titration.

A series of 26 rats was also injected with bile salts. Doses comparable with those used with guinea pigs usually caused death within 24 hours, but in no case was ulceration similar to that produced in guinea pigs observed. Occasionally slight, superficial

[7] Birch, T. W., Harris, L. J., and Ray, S. N., *Biochem. J.*, 1933, **27**, 590.

erosions were seen, but nothing comparable with the deep, extensive ulcers which developed in the guinea pigs was observed. When the rats' adrenals were stained with silver nitrate, the darkening was immediate and marked. At present we are unable to say definitely whether this resistance of the rat to ulcer production through bile salt injection is due to their ability to synthesize vitamin C or to some other species difference.

7544 P

Differential Reduction of Methylene Blue by Living Organisms.

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With the oxidized dye and with a leucobase prepared by adding small amounts of HCl and $Na_2S_2O_3$ to dye solutions axial differentials or gradients in rate of reduction of dye have been observed in various unicellular and multicellular organisms. *Paramecium* is able to reduce methylene blue in mixtures of culture fluid and dye exposed to air, provided the animals are numerous in proportion to volume of fluid or gather in aggregations and decrease oxygen locally, or provided other organisms which take up oxygen are present, but reduces more rapidly in sealed preparations with small amounts of fluid. With high oxygen content of solutions the anterior ectoplasm stains more rapidly than other parts. In low concentrations of dye with somewhat lower oxygen content permitting some reduction stain appears first in the deepest part of the posterior entoplasm, extends anteriorly in the entoplasm and the ectoplasm does not stain or stains more slowly, also from posterior to anterior, except for the extreme posterior tip which often stains less rapidly than adjoining regions. Apparently rate of staining under these conditions varies inversely as reducing power of different regions. In stained but uninjured animals reduction first becomes evident in the ectoplasm of the anterior end and progresses posteriorly, somewhat more rapidly along the peristome than on the aboral side and the extreme posterior tip of some individuals shows early reduction. In high concentrations the anterior ectoplasm stains more rapidly and more deeply than other regions at first and with sufficient staining it is injured and its reducing power is decreased or lost while more posterior regions are still able to reduce the dye in low oxygen.