the time of irradiation in relation to EP injection. There is maximum sensitivity to irradiation when it is given 3 hours before or 24 hours after EP. Radiosensitivity is at a minimum when irradiation is given one to 3 hours after EP injection. Fractional depression of erythropoiesis by varying doses of gamma rays is less in animals injected with EP, when it is given before irradiation. There is no effect of EP on fractional depression produced by irradiation given 6 hours before EP.

1. Eskuche, I., Hodgson, G., Proc. Soc. Exp. Biol. and Med., 1965, v119, 1102.
2. Stohlman, F., Jr., ibid., 1961, v107, 751.
3. Porteus, D.S.C.T.S.O., Hirashima, K., Lajtha, L. G., Nature, 1965, v206, 204.

Received January 31, 1966, P.S.E.B.M., 1966, v122.

Neutralization of Alpha-Staphylotoxin Cytotoxic Effect with Human And Monkey Immune Globulins. (31157)

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The data presented by Elek(1), Bernheimer(2), and Rogers and Melley(3) attest that the staphylococcal α toxin (SAT) is lethal, hemolytic, and leukocidal. Arenstein *et al*(4), Kienitz and Schmelter(5) and Cooper *et al*(6) reported that SAT is cytotoxic for monkey kidney tissue and human amniotic cell cultures. Thal and Egner(8) and Thal *et al*(9) observed very rapid cell damage in cultures of human esophageal epithelial cells and of rabbit fibroblasts. This activity reveals itself in alteration of the permeability of the cell membrane, swelling and vacuolization of the protoplasm, and nuclear changes, followed by cytolysis. Results with other cell lines were summarized by Korbecki and Jeljaszewicz(7). The outcome of the experiments varied. Hyperimmune sera employed in the neutralization tests often contained preservatives which interfered with the results (Kienitz and Schmelter(5)). It was postulated that a modified experimental technique may offer a better insight into the mechanism of toxin-antitoxin relationship.

Antibodies against SAT are being measured principally by the inhibition of the SAT hemolysin. The tissue culture cell damaging factor of SAT, however, is not identical with the α hemolytic principle (Bernheimer(2)). Experiments were set up to investigate further the possible relationship of these two factors by measuring the cytotoxic activity neutralizing property (CANP) of human sera and comparing the results with those of anti-SAT hemolysin determinations.

It had been noted that not only persons afflicted with clinically manifest staphylococcal disease but also the majority of adults without a history of staphylococcal infection have measurable antistaphylococcal antibodies in their sera (Elek(1)). To establish whether man is the only primate endowed with such circulating antibodies, parallel experiments were carried out with selected human and non-human primate sera.

It was also assumed that an inquiry into the role of the various immune globulins in the CANP of the sera (if any) might yield revelant results.

Materials and media. All materials were kept at $-20°C$ without a preservative.

Staphylococcal alpha toxin (SAT) was prepared by one of us (O.F.) at the Walter Reed Army Institute of Research in 1964 according to the method of Lominski and Arbuthnott(10) by methanol and Sephadex G75 fractioning from *S. pyogenes* strain S6. It contained 1.3% carbohydrate and 0.02% P. It was inactivated by trypsin. The molecular weight was 41,000. Immunoelectrophoresis at pH 6.5 revealed a strong 3 S and a weak 16 S component. The MHD for rabbit RBCs was 80,000/mg material; the LD_{50} 3 μg/20

g mouse. The toxin was lyophilized and kept at −20°C.

Sera. Pools of sera from 27 apparently healthy American adult males, 32 healthy Thai soldiers, 20 Americans and 21 Thais in Thailand who had staphylococcal food poisoning 5 to 10 days before blood was collected from them, and 24 Americans and 23 Thais with chronic staphylococcal skin lesions were examined as well as pools of sera from 18 vervets (African green monkeys, *Cercopithecus aethiops*), 7 patas (*Erythrocebus patas*) and 10 rhesus monkeys (*Macaca rhesus*). The non-human primates were adults of both sexes in apparently good health. Sera from 2 each of infant vervet, patas and rhesus monkeys were also available.

Whole sera, as well as immune globulins prepared from them according to the method of Vaerman *et al*(11) were used. The immune globulins were IgG (γ_2), IgA (γ_{1A} or β_{2A}) and IgM (β_{2M}). These globulin fractions were tested for purity after agar gel immunoelectrophoresis against specific antiglobulin sera. The amount of IgG in whole sera and in purified preparation was determined according to Claman and Merrill(12). Purified IgG was obtained also by separation on DEAE-Sephadex, chloride form, at pH 6.5, according to Baumstark *et al*(13).

All tests were carried out in triplicate.

Antihemolysin titration was performed by the modified method of Kienitz and Kümmel (Felsenfeld and Nasuniya(14)).

Precipitating antibodies were identified by immunoelectrophoresis of the serum pools in 0.85% Ionagar (Oxoid Corp., London, England) for 2 hours at 2.25 V/cm and veronal buffer at pH 8.6. The troughs on one side were filled with SAT, on the other with the respective antiprimate rabbit sera to identify the precipitation lines.

Cytotoxic activity neutralizing potency (CANP) of the sera was estimated in human embryonic kidney tissue cultures (Flow Laboratories, Inc., Rockville, Md.). Tube cultures, with the Eagle (Earle) basic maintenance medium containing 2% calf serum but without antibiotics, were used. The maintenance medium was renewed the day before the test, and the tissue cultures incubated at 37°C. At the same time increasing amounts of SAT, from 0 to 2 mg, were dissolved in maintenance medium. No immune globulin or 5 mg were added to each such solution, and the mixtures kept at 2°C until the next day. In another series whole serum diluted with the maintenance medium to contain 5 mg IgG/ml was used instead of the purified immune globulins. On the day of the experiment the maintenance medium was drained off the tissue culture tubes and replaced by 1 ml aliquots of this medium containing SAT and immune globulin, or SAT and whole serum, or control solutions. Stationary incubation at 37°C was used, and the tubes were examined daily for 3 consecutive days.

For comparison, tubes inoculated with *Mycoplasma pneumoniae* and SAT, in varying dilutions, were used.

Results. The antihemolysin activity of the sera of patients after or with staphylococcal infections was stronger than that of apparently healthy persons. Healthy Thais showed higher titers than Americans without apparent staphylococcal infection.

SAT hemolysin neutralizing activity and CANP were observed also in the tests with pattern of young monkeys.

Precipitating antibodies along lines identical with IgG and IgA appeared in all tests with serum pools except in those with the sera of infant monkeys. Precipitation along the IgM line fluctuated in intensity. It was stronger in tests with the sera of Thais but did not appear in the immunoelectrophoretic pattern of young monkeys.

The 50% cytotoxic activity (CPE_{50}) of the SAT employed in the present experiments was 0.37 ± 0.4 mg. The cytopathogenic effect is shown in Fig. 1. None of the control tissue culture tubes (with sera or globulins but without SAT as well as those containing only tissue cultures without any addition) showed such changes. Fig. 2 shows a negative control. The effects of *Mycoplasma pneumoniae* in 1:50 dilution and of *Mycoplasma pneumoniae* 1:50 together with 0.6 mg SAT are presented in Fig. 3 and 4, respectively.

None of the infant monkey serum pools

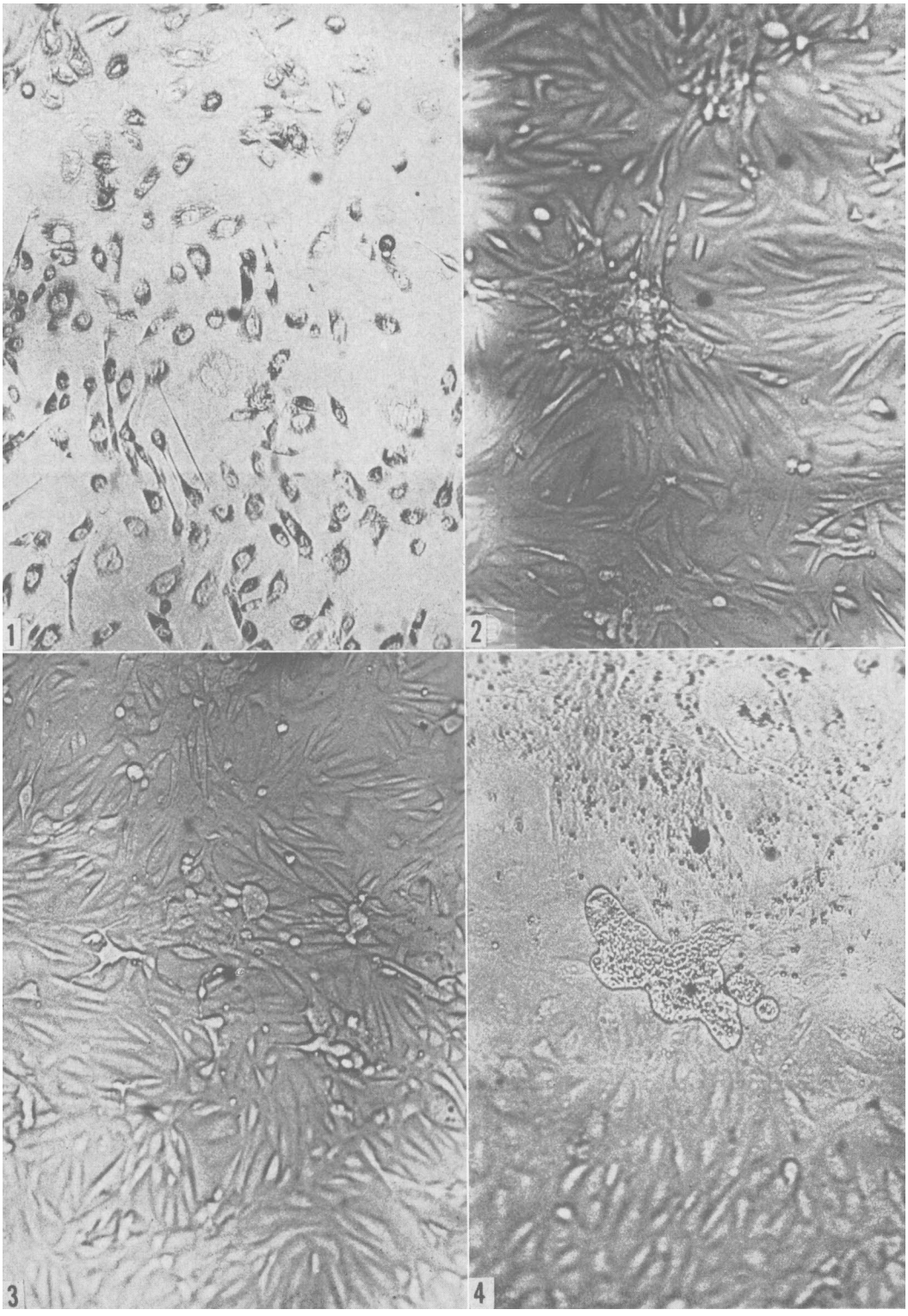
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FIG. 1-4. CPE in HEK cell cultures. AFIP photograph #134. ×387.
FIG. 1. Staphylococcal α toxin 0.6 mg.
FIG. 2. Uninoculated culture.
FIG. 3. *Myco. pneumoniae* 1:50.
FIG. 4. Staphylococcal α toxin 0.6 mg and *Myco. pneumoniae* 1:50.

showed CANP. Neither did these sera neutralize SAT hemolysin.

Neither IgA nor IgM from adult human or monkey serum pools neutralized 1 CPE_{50} in 5 mg doses. The CANP of the whole sera (when measurable) was the same as that of the IgG fractioned from the same pool (Table I).

Discussion. IgA and IgM from human and monkey sera did not show CANP when tested under these experimental conditions while these globulins interacted with antigen to form a precipitate. The results presented in Table I indicate that CANP against SAT in human embryonic kidney tissue cultures is principally bound to IgG.

Thais, who live in a tropical country where staphylococcal infections prevail, have higher titers of staphylococcal toxin neutralizing antibodies than inhabitants of the United States. This has been reported previously when antihemolysin, antienterotoxin and toxin precipitating antibodies but not CANP were studied in samples of these two populations (Felsenfeld and Nasuniya(14)). This was borne out also by the results of the precipitation reactions.

TABLE I.

Serum pool	Units of SATH* neutralized	CPE_{50} neutralized: Whole† serum	CPE_{50} neutralized: IgG
Human‡			
Healthy males			
US	.8 ± .3	1.4 ± .3	1.1 ± .2
Thai	1.8 ± .4	1.6 ± .4	1.2 ± .3
Staph food poisoning			
US	2.9 ± .4	1.5 ± .3	1.6 ± .2
Thai	3.4 ± .3	1.7 ± .2	1.4 ± .3
Chronic staph skin lesions			
US	2.9 ± .4	3.6 ± .6	3.2 ± .2
Thai	4.1 ± .5	4.6 ± .7	4.2 ± .4
Other primates‡			
Vervet	1.3 ± .2	5.2 ± .6	4.8 ± .5
Patas	1.7 ± .4	4.2 ± .4	4.0 ± .4
Rhesus	1.9 ± .2	3.6 ± .4	3.8 ± .3

* Staphylococcal α toxin hemolysin, whole serum only.
† Containing 5 mg IgG per ml tissue culture tube.
‡ Adults only.

SAT antihemolysin, precipitation and tissue culture CANP were present in adults who had no history of staphylococcal disease. These immunological reactions are probably due to clinically inapparent or inapparent low grade infections in man (Elek(1)). Our observations indicate that similar conditions exist in monkeys. Contact with other primates including man is the probable cause of the appearance of antistaphylococcal antibodies in monkeys kept in captivity even though their history may not reveal clinically manifest disease.

The CANP levels were higher in staphylococcal skin lesions than in apparently normal persons and in individuals who had recently recovered from staphylococcal food poisoning. SAT hemolysin neutralization titers, however, were evaluated also in that type of staphylococcal disease. It is doubtful if SAT plays a role in food poisoning but it has a tissue damaging factor. One may assume, therefore, that increased CANP is a more specific indicator of chronic or repeated staphylococcal tissue damage than elevated antihemolysin titers.

Summary. Staphylococcal α toxin produced cytopathogenic effect in human embryonic kidney tissue cultures. This effect could be neutralized with whole serum and by the IgG but not by the IgA and IgM fractions of human and other primate sera, whereas these immune globulins interacted with antigen to form a precipitate. The neutralizing potency of the sera was more pronounced in persons with chronic staphylococcal skin lesions.

The authors wish to thank Major E. D. Shaw, Chief, Virology Section, Armed Forces Institute of Pathology, Washington, D. C. for the microphotographs, and for the control experiments with *Mycoplasma*; Flow Laboratories, Inc., Rockville, Md. for their generous gift of tissue cultures; the authorities of the Walter Reed Army Institute of Research,

Washington, D. C., for staphylotoxin; and Drs. Wm. Greer and T. C. Orihel at this Center for providing monkey sera.

1. Elek, S. D., *Staphylococcus pyogenes* and Its Relation to Disease, Livingstone, Cambridge, 1959.
2. Bernheimer, A. W., Ann. N. Y. Acad. Sci., 1965, v128, 112.
3. Rogers, D. E., Melley, M. A., ibid., 1965, v128, 274.
4. Arenstein, M. D., Madoff, M. A., Weinstein, L., Yale J. Biol. Med., 1963, v35, 373.
5. Kienitz, M., Schmelter, G., Zentralbl. Bakt., Abt. I., 1964, v193, 447.
6. Cooper, L. Z., Madoff, M. A., Weinstein, L., J. Bacteriol., 1964, v87, 127.
7. Korbecki, M., Jeljaszewicz, J., Zentralbl. Bakt., Abt. I., 1963, v192, 430.
8. Thal, A. P., Egner, W. E., J. Exp. Med., 1961, v113, 67.
9. Thal, A. P., Kitamura, S., Hatafuku, T., Wolf, P., Pearson, B., in Cell Culture in the Study of Bacterial Disease, M. Solotorovsky, ed., Rutgers Univ. Press, New Brunswick, N. J., 1963, p39.
10. Lominski, I., Arbuthnott, J. P., Spence, J. B., J. Path. Bact., 1963, v86, 258.
11. Vaerman, J. P., Heremans, J. F., Vaerman, C., J. Immunol., 1963, v91, 7.
12. Claman, H. M., Merrill, D. J., Lab. Clin. Med., 1964, v64, 685.
13. Baumstark, J. S., Laffin, R. J., Bardwall, W. A., Arch. Biochem. Biophys., 1964, v108, 514.
14. Felsenfeld, O., Nasuniya, N., J. Trop. Med. Hyg., 1964, v67, 300.

Received February 2, 1966. P.S.E.B.M., 1966, v122.

Mortality and Histopathology of Germ-Free Rats and Mice Exposed To 100% Oxygen.* (31158)

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Many workers have noted similarities in the histology of lungs from animals exposed to hyperoxia and from those suffering from various pulmonary diseases(1-5). Rats and mice are known to be chronically afflicted with murine pneumonia and related diseases which can cause thickening of the alveolar wall, atelectasis, edema, lymphoid invasion, etc., much the same as in oxygen toxicity(6). In fact, the oxygen toxicity syndrome has been referred to as oxygen pneumonitis(7). The extent to which such chronic infections contribute to animal mortality in oxygen toxicity or at least to its pulmonary histopathological aspects have never been fully determined, although attempts have been made to reduce the problem by use of Caesarian derived animals(8) and to define the nature of the infection by culture of the lungs of afflicted animals(1-5,9).

Although one might expect pulmonary disease to heighten susceptibility to O_2 toxicity, our general impression from work with rats is that those with chronic respiratory conditions tended to live longer. Our preliminary attempts to culture lungs of rats which died of oxygen poisoning resulted in finding certain entero-bacteria which might be called opportunists that migrate to areas of least resistance in the body. Others, however, have found no bacteria in the lungs of guinea pigs and rats exposed to oxygen for varying periods of time(1-5,9). It seemed to us that an alternate and perhaps more direct method of delineating the role of chronic infection in oxygen toxicity would be to study the response of germ-free animals to hyperoxia.

Materials and methods. Two germ-free experiments have been accomplished involving a total of 8 rats and 14 mice breathing 99 ± 1% oxygen at one atmosphere (OAP). Germ-free Sprague-Dawley rats, about 200 g in weight, and germ-free adult white mice were obtained from the germ-free colonies bred and maintained by Laboratory Animal Facilities of Ohio State University. For exposure to OAP, they were transferred directly *via* locks into a peracetic-acid-sterilized poly-

* Supported by NASA grant NsG 295-62.