caused an absolute deficiency of glucagon itself, a suppression of the hormone at source, wherever this may be. An absolute deficiency of a hormone is frequently reflected by an increased sensitivity to that hormone(7). The exaggerated physiologic response to a small dose of glucagon fits in with an absolute deficit of this hormone produced by the drug. We have found by contrast that exposure to the drug causes no significant change in the body's responsiveness to insulin. This is also in contrast with the improvement in insulin sensitivity observed in obese diabetics after adequate dietary treatment alone(7).

The speed with which the glucose lowering effect of the drug is established (10 minutes) and reversed suggests reversible interplay of enzyme systems. The anatomical integrity of the alpha cells after 70 days of intensive exposure does not support the original claim of the German workers that the drug functions by destruction of the alpha cells.

Conclusions. 1. A simple method for gauging changes in the output of glucose by the liver has been described. Throughout postabsorption, there are present in hepatic venous blood aperiodic fluctuations in glucose which are reflected throughout the major peripheral arterial tree. Under the influence of the drug, these fluctuations at both sites promptly disappear. The quantitated changing rate of output of glucose by the liver after Orinase (33% fall in 10 minutes) is drastic. A small dose of glucagon by vein immediately reverses this trend with a return in exaggerated form of the normal postabsorptive fluctuations. As a corollary one may infer that long use of the drug does not impair liver glycogen deposition. 2. It is suggested that the site of action of Orinase is proximal to the phosphorylase enzyme systems and takes the form of a suppression of glucagon production at source with a resulting reduction of glucose output by the liver. After 70 days of intensive exposure to the drug in the normal dog, acute signs and symptoms of toxicity are present but no histopathologic changes in the liver, kidneys, thyroid, adrenals or pancreas (including alpha-beta cell ratios). 3. No evidence was found to suggest that the drug works by destruction of the alpha cells of the pancreas.

1. Bondy, P. K., *J. Clin. Invest.*, 1952, v31, 231.
2. Anderson, G. E., Hillman, R. W., Van Elk, I. F. A., and Perfetto, A. J., *Am. J. Clin. Nutrit.*, in press.
3. Anderson, G. E., *Science,* 1955, v122, 457.
4. Nelson, H., *J. Biol. Chem.*, 1944, v153, 375.
5. Mirsky, I. A., *Metabolism,* 1956, v5, 138.
6. ———, *Ciba Foundation Colloquia on Endocrinology, London,* 1953, v6, 263.
7. Anderson, G. E., *Diabetes,* 1954, v3, 462, 466.

Received May 7, 1956. P.S.E.B.M., 1956, v92.

Pulmonary Vascular Changes in the Mouse During Anaphylactic Shock.* (22472)

LEON S. KIND AND JAMES I. GALLEMORE. (Introduced by William M. McCord.)
Department of Bacteriology, Medical College of South Carolina, Charleston.

Discussions of anaphylaxis generally emphasize the differences in reactions in various species of animals. However, Burrage and Irwin, by direct microscopic observation of the living pulmonary circulation, have shown a marked similarity in changes occurring during anaphylaxis in the rabbit and the guinea pig(1). They noted pulmonary constriction, clumping of red cells and embolization in both species. This article will describe pulmonary vascular changes in the mouse during anaphylactic shock. The methods employed in observing pulmonary circulation were essentially the same as those of Burrage and Irwin(1).

* This research was supported by N.I.H. Grant E 372.

TABLE I. Pulmonary Vascular Reactions in Pertussis-Inoculated Horse Serum-Sensitized Mice after Challenge Doses of Horse Serum.

Mice sensitized with	Mice challenged with	No. of mice reacting* /Total No. challenged	% reacting	No. of mice with emboli /Total No. reacting	% showing emboli
.1 ml pertussis vaccine + .05 ml horse serum, i.p.	.2 ml horse serum, i.v.	37/53	70	19/37	51
.1 ml pertussis vaccine, i.p.	*Idem*	0/15	0	0	0

* Arteriolar constriction, clumping of red cells, or embolization.

Materials and methods. Female mice† weighing 17 to 25 g were sensitized by intraperitoneal injection of 0.1 ml of *Hemophilus pertussis* vaccine and 0.05 ml of horse serum. Challenge doses of horse serum (0.2 ml) were administered 10 to 20 days later via the intravenous route. Ability of *H. pertussis* to increase sensitivity of mice to active or passive anaphylaxis has been well established(2-4). The lung was exposed in the following manner: the animals were anesthetized with tail vein injections of 50-60 mg/kg of pentobarbital sodium; the 27 gauge needle employed for injecting the anesthetic was not removed from the vein and additional amounts of pentobarbital sodium were administered whenever necessary; a tracheotomy was performed and a 20 gauge needle was inserted; the needle did not fill the tracheal lumen thus allowing gases returning from the lung to escape through the opening in the trachea as well as through the animal's nose and mouth; the flow of oxygen was adjusted until respiratory movements ceased; the mouse was then turned to the left lateral position and tied down securely to the animal board; an incision was made at the level of the xiphoid process extending from vertebral column to the costal angle; vessels were ligated and muscles were divided down to the thoracic cage; a small puncture was made between fourth and fifth ribs into the pleural cavity and oxygen flow was adjusted so that minimal collapse of the lung occurred; the initial puncture wound was then extended dorsally to the sacrospinalis group of muscles, and the fourth rib was ligated and excised exposing the middle lobe of the lung. The oxygen flow was then increased in order to allow the lung to expand and fill the thoracic cage. Only a minimal amount of blood should be lost in this whole procedure. Following exposure of the lung, the animal was placed on a stand and the edge of the lung was transilluminated by the quartz rod method of Knisely(5). Light from a 500 watt projection bulb was transmitted through a fused quartz rod similar to the one described by Hoerr(6). Ringer solution at 38°C flowed out from the tip of the rod and the edge of the lung rested on this stream of Ringers. Microscopic observations were made through a Leitz binocular stereoscopic microscope using a 12X lens and 12X wide field oculars. The microscope was mounted on a special adjustable stand (American Optical) and motion pictures were taken with a Cine Kodak Special camera mounted on an adjustable tripod.

Results. Pulmonary blood flow was noted before and after an intravenous challenge dose of horse serum. The data in Table I list the observed changes. Of 53 sensitized mice, 37 (70%) showed at least one of the following phenomena after the challenge dose of horse serum: (a) sludge (clumping of the red cells); (b) arteriolar constriction; and (c) embolization. Embolization occurred in 19 (51%) of the reacting mice. In most cases the emboli were small and multiple, but in 5 instances a large single embolus which filled the lumen of a 50-150 mμ pulmonary arteriole was seen to shoot by. We were fortunate enough to obtain a film of one of these large emboli. Of 15 non-sensitized mice, *i.e.* mice injected with only pertussis vaccine, none showed any of the changes which were observed in sensitized animals after challenge

† From Tumblebrook and Carworth Farms.

doses of horse serum. It seems unlikely that the emboli observed in sensitized mice were air bubbles. Air emboli were observed in mice injected via the tail veins with saline containing air bubbles. Air emboli have thick, clearly visible refractile outer boundaries. In contrast, the outer edge of the embolus seen in anaphylactic animals was not at all distinct. In addition, air emboli were visible in the pulmonary circulation immediately after the injection of an air bubble into the tail vein. The emboli in anaphylactic mice did not appear until a few minutes after the challenge dose of antigen had been administered.

It should be mentioned that in the majority of cases administration of challenge doses of horse serum was not followed by death of the animal. Vasoconstriction, embolization and sludge were observed, but conditions returned to normal in a few minutes. In contrast, challenge doses of horse serum invariably killed 80 to 100% of pertussis-inoculated horse serum sensitized mice which were not subjected to the procedures of anesthesia, exposure of the lung and oxygen insufflation. It seems likely that the combination of anesthetic and oxygen had a protective effect in mouse anaphylaxis.

Discussion. Our observations of pulmonary vascular changes in mice during anaphylactic shock are very similar to the observations in rabbits and guinea pigs reported by Burrage and Irwin(1). Our results are also consistent with those of McMaster and Kruse(7) who described vascular changes in the ears of mice during anaphylaxis. The latter noted vascular spasm, clumping of cells and plasma skimming. It is possible that the pulmonary phenomena observed during mouse anaphylaxis account for the symptoms observed in the intact animal, namely cyanosis and respiratory embarrassment. At present the nature of the pulmonary emboli which have been seen is unknown. Our current hypothesis is that they are clumps of leucocytes sticking to an antigen-antibody precipitate.

Summary. Arteriolar constriction, clumping of red cells, and embolization have been noted in the pulmonary circulation of mice during anaphylactic shock.

We are indebted to Dr. Melvin H. Knisely for his encouragement and his many helpful suggestions.

1. Burrage, W. S., and Irwin, J. W., *J. Allergy,* 1953, v24, 289.

2. Malkiel, S., and Hargis, B. J., PROC. SOC. EXP. BIOL. AND MED., 1952, v80, 122.

3. Kind, L. S., *J. Immunol.,* 1953, v70, 411.

4. Munoz, J., Schuchardt, L. F., and Verwey, W. F., *Fed. Proc.,* 1954, v13, 407.

5. Knisely, M. H., *Anat. Rec.,* 1936, v64, 499.

6. Hoerr, N. L., *Medical Physics,* The Year Book Publishers, Inc., Chicago, Illinois, 1950.

7. McMaster, P. D., and Kruse, H., *J. Exp. Med.,* 1949, v89, 583.

Received May 11, 1956. P.S.E.B.M., 1956, v92.

Sustained Propagation of Sarcoma 180 in Tissue Culture.* (22473)

G. E. FOLEY AND B. P. DROLET. (Introduced by Sidney Farber.)

Laboratories of Microbiology, Children's Cancer Research Foundation, and Department of Pathology, Harvard Medical School, Children's Medical Center, Boston, Mass.

The delineation of the specific amino acid, salt and vitamin requirements of a mouse fibroblast (strain L) and of a human carcinoma (strain HeLa)(1-6) has permitted the continuous propagation of these cells in a medium containing only demonstrably essential growth factors, in which the omission of a single amino acid or vitamin, of glucose or serum protein, resulted in the cessation of growth and cell death. Eagle also has reported the successful isolation and propaga-

* This investigation was supported in part by research grants C-937 and C-2782, from National Cancer Institute of N.I.H., U.S. Public Health Service.