sometimes sheets of them have absorbed some of the stain. Cells around traumatized areas also are often stained. The foci of proliferation in virus lesions, however, stand out prominently as more or less isolated clumps of well stained cells which are easily recognized from their distinct focal arrangement.

Most of our observations have been made with the lesions of the St. Louis type of encephalitis virus in which it is usually possible to recognize the virus foci in the vitally stained membrane in 18 hours and always in those 24 hours old. The focal lesions from vaccinia and variola virus also are well stained by trypan blue and easily recognized. The finer cell structure of the virus lesion is not easily made out in the thickened membrane, but the cells show well in paraffin imbedded sections.

11161 P

A Consideration of Extra-Valvular Elements in the First Heart Sound.

JOHN R. SMITH, WM. B. KOUNTZ AND ARTHUR S. GILSON.

From the Departments of Internal Medicine and Physiology, Washington University School of Medicine, and the Barnes Hospital, St. Louis.

The relation between auricular systole and the first sound, the rôle of the atrio-ventricular valves, and myocardial contraction have been considered, together and separately, as factors contributing to the production of the first heart sound.[1-7] Some observers have felt that closure and tensing of the atrio-ventricular valves at the onset of systole are the sole elements in causing the first sound, while others have adhered to the theory that the initial heart sound is partly muscular and partly valvular in origin. We investigated the problem, applying a method designed to prevent atrio-ventricular valve movement.

The instrument here used to record the heart sounds was a cathode-ray stethograph, furnished by the Burdick Corporation. The instrument is aperiodic to most vibrations produced by the heart, and the

1 Eckstein, R. W., *Am. J. Physiol.*, 1937, **118**, 359.
2 Wiggers, C. J., and Dean, A. L., *Am. J. Physiol.*, 1916, **12**, 476.
3 Wiggers, C. J., *Arch. Int. Med.*, 1919, **24**, 471.
6 Palfrey, F. W., *New England J. Med.*, 1929, **200**, 1917.
7 Dock, W., *Arch. Int. Med.*, 1933, **51**, 737.

lags and overshoots, so characteristic of most string galvanometers used for this work, are lacking. Therefore, the sound tracings are more nearly exact reproductions of the cardiac vibrations produced. The instrument is so constructed that sound waves below fifty cycles per second, which are not appreciated by the normal ear, may be heard.

Dogs were anesthetized with veterinary nembutal. The thoraces were opened and respiration was maintained by a respiratory pump. To prevent movements of the mitral and tricuspid valves, small balloons were affixed to the ends of brass tubes 3 mm in diameter; these were introduced into the ventricular cavities through small openings made in the auricles and then through the mitral and tricuspid apertures. The inflow of blood into the heart was then cut off by clamping the venae cavae, so as to empty the heart of blood, and the balloons were inflated by water or air to a pressure of 70 mm of Hg against a manometer. While this inhibited all movement of the auriculo-ventricular valves, the myocardium remained intact and could contract against the resilient balloons. Sounds arising from both ventricles were recorded by applying the transmitter of the stethograph directly to the myocardium at the intraventricular septum just superior to the apex. Auscultation was also done by placing an ordinary stethoscope on the myocardium. Small doses of barium chloride were administered to these hearts to forestall ventricular fibrillation.

Control readings with simultaneous electrocardiograms were obtained on each animal before the experimental procedures were begun. When the "intraventricular" balloons were in place and inflated, the character of the sounds was profoundly changed, but systolic vibrations were recorded, and were heard on direct auscultation. It must be remembered that the inflated balloons filled the ventricular cavities without visibly distending them. The first sound on auscultation had lost much of its "normal" quality, but persisted as a dull snapping sound during the time the balloons were inflated.

Constriction of the auriculo-ventricular ring was carried out by drawing as tightly as possible a ligature passed around the auriculo-ventricular sulcus as done by Dock;[7] this prohibited any flow of blood into or out of the ventricles and immobilized the atrio-ventricular valves. In a vigorously beating heart in good condition, systolic sounds could be heard during the period of auriculo-ventricular constriction, and were very similar to those obtained when the intraventricular balloons were used. They were dull, snapping sounds. The second heart sound, of course, completely disappeared. In some hearts—those that had been subjected to previous manipulation, and in which the myocardium may have been weakened or damaged—

constriction of the auriculo-ventricular ring caused a cessation of all heart sounds. Dock stated that constriction of the auriculo-ventricular ring invariably caused complete cessation of the heart sounds. Our results, therefore, were only in partial agreement with his.

During the onset of ventricular systole it would seem that, sudden change of intraventricular pressure would tense all structures subjected to the pressure. In the vigorously contracting myocardium this tensing is such as to produce audible sounds in the absence of motion of the ordinarily movable valve structures.

11162 P

Anesthetized Kittens Used to Test for Staphylococcus Enterotoxin.

N. M. PHATAK AND C. F. PENTLER. (Introduced by K. F. Meyer.)

From the George Williams Hooper Foundation, and the Department of Pharmacology, University of California Medical School, San Francisco.

The reaction of kittens to staphylococcal enterotoxin was described by Dolman, Wilson and Cockcroft,[1] and confirmed by Kupchik[2] and Minett.[3] It was shown that kittens will vomit after intraperitoneal injection of heated staphylococcal filtrates that contain enterotoxin.

We find that this test can be rendered more objective and more convenient by anesthetizing the kittens with sodium pentobarbital. The kittens are given a small meal; after 30 minutes they are injected intraperitoneally with sodium pentobarbital. The dosage is 30 mg per kg body weight, as a 5% aqueous solution. This produces light anesthesia, which lasts for several hours. The heated staphylococcal filtrates are injected intraperitoneally after the onset of anesthesia; then the kittens are laid out in a row and observed for vomiting. Only definite vomiting with expulsion of vomitus, between 20 and 90 minutes after injection of the filtrate is considered as positive.

The kittens should be kept warm, and turned over every hour to avoid hypostatic congestion. It is easy to handle a large number of kittens in this manner. Sensitivity is somewhat reduced, but specificity may be increased since the effects of excitement are avoided. It is important to have shallow anesthesia, and to use 50% larger doses of filtrates than when working with conscious kittens.

1 Dolman, Wilson and Cockcroft, *Canad. Pub. Health J.*, 1936, **27**, 489.

2 Kupchik, *J. Infect. Dis.*, 1937, **61**, 320.

3 Minett, *J. Hyg.*, 1938, **38**, 623.