where R is the gas constant, T the absolute temperature, N Avogadro's number, r the radius of the particle and $\eta$ the viscosity of the medium. This gives the diameter of the hydrated particle which will be appreciably higher than the unhydrated particle. To bring the size, as calculated from diffusion measurements, in line with that measured from electronmicrographs of the C.F. antigen of influenza the diffusion constant of the rabies C.F. antigen was interpolated into a curve relating the diffusion constants with particle radii. These are calculated from the molecular weights of the different proteins in their dehydrated states (Polson)(5). This curve is shown in Fig. 2. When the diffusion constant of the C.F. antigen of rabies is interpolated into this curve a particle radius of $6.2 \times 10^{-7}$ cm is obtained. This gives a value for the diameter of the particle of 12.4 m$\mu$.

*Ultracentrifugation of the rabies soluble antigen.* From ultracentrifugation experiments in which the swinging bucket type of rotor of the Spinco ultracentrifuge was used a sedimentation constant of approximately 25 Svedberg units was found. This value corresponded very well with that calculated for a protein particle of 12 m$\mu$ diameter. These centrifugation experiments, however, cannot be regarded as final as it was difficult to maintain a constant rotor temperature at velocities >30,000 rpm, and the calculations of the sedimentation constant had to be made on runs at lower speeds where only small boundary displacements took place, but during which the rotor temperature remained constant at 7°C.

*Summary.* 1. A particle size of 12 m$\mu$ has been determined by a method of diffusion coupled with biological assay for the soluble antigen of rabies extracted from infected suckling mouse brains. This value agrees with that calculated from the approximate sedimentation constant calculated from the sedimentation rate in the swinging bucket rotor of the Spinco ultracentrifuge. 2. No conclusions regarding the homogeneity of the soluble antigen can be drawn at this stage.

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## Survival of Dogs with Bilateral Phrenectomy and Extensive Transection of Intercostal Nerves. (20633)

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According to Brown and Satinsky(1) dogs invariably die after destruction of both phrenic nerves. Partly on this basis they claimed functional restoration of the diaphragm by uniting the vagus in the thorax with the distal end of a severed phrenic nerve. After a suitable interval following such an anastomosis, dogs tolerated section of the remaining phrenic nerve and fluoroscopic studies seemed to indicate that the vago-phrenic anastomosis was responsible for rhythmic contraction of the diaphragm. On the other hand, there are numerous reports antedating that of Brown and Satinsky claiming a high degree of tolerance to section of both phrenic nerves in man and other mammals, including the dog. Bilateral phrenectomy in man without untoward results has been reported by Greene(2), Angle (3), Campbell(4), and according to Lemon (5) by many others. The effects of destroy-

ing both phrenic nerves have been studied in the rabbit, dog, goat, horse and monkey by Hare and Martin(6); in the dog by Lemon (7), Jappeli(8), and Feldman and Morrison (9). All of these authors agree that animals so treated survive the procedure without marked respiratory distress, provided they are not very young. Hare and Martin found that young rabbits die when both phrenic nerves are cut, and similar results were reported for kittens by Coombs and Pike(10). However, Coombs and Pike also found that mature cats tolerate the procedure very well. According to Lemon(7) not only will dogs tolerate bilateral phrenectomy but section of most of the intercostal nerves in addition.

Although the earlier reports seem convincing, the experience of Brown and Satinsky led us to do a series of bilateral phrenectomies in dogs. Since it was soon apparent that dogs do survive section of both phrenic nerves, the work was extended to determine the effects of still further reduction in respiratory musculature.

*Methods.* Eight grown but young dogs were subjected to section of both phrenic nerves under nembutal anesthesia and with aseptic precautions. In 6 animals the phrenic nerves were severed in the neck, and in 2 these nerves were cut 1 cm above the diaphragm. After some days or weeks several of the dogs were subjected to further reduction in the number of active respiratory muscles by cutting intercostal nerves, and in one instance by removal of possible accessory muscles and spinal cord transection. When finally sacrificed, the chest was opened in each case and the phrenic nerve remnants were strongly stimulated with induction shocks. Following stimulation, a portion of each nerve was taken distal to the point of original section and placed in 1% osmic acid. The osmicated pieces of nerve were examined microscopically for any signs of intact fibers in carefully teased preparations.

*Results.* All dogs, except the first which was lost during the operation, survived bilateral phrenectomy without difficulty. Immediately after section of the second phrenic nerve, each dog exhibited a pronounced increase in costal movements. This increase became progressively less noticeable during the first week and did not change appreciably thereafter.

The first dog to be sacrificed, Dog 2, had lived uneventfully for 5 weeks following cutting the phrenic nerves. The animal was anesthetized and then subjected to the following in succession: transection of the spinal cord between the 10th and 11th thoracic arches, ligature of the axillary vessels and section of the roots of the brachial plexus, section of the long thoracic nerve, transection of the scalene and infrahyoid muscles, cutting of all but the upper 2 intercostal nerves near their origins on the right side, cutting all but the upper 4 intercostal nerves on the left side. While working on the left fourth intercostal nerve the pleura was punctured and the dog expired after a few gasping movements. Until the pleura was inadvertently opened this animal was breathing heavily with the remaining intercostal muscles but showing no signs of respiratory distress. Since this experiment indicated an enormous reserve of respiratory musculature in phrenectomized dogs, the other animals were subjected to different types of reduction in their intercostal musculature and permitted to live. It was established that a phrenectomized dog will live after either the upper or the lower 6 intercostal nerves have been severed bilaterally, when all intercostal nerves are cut along the mid-axillary line, and when only the 3 upper pairs remain. One dog lived for 4 hours with only the lower 6 intercostal nerves of one side intact.

Complete phrenic section was confirmed in all animals by faradization of the distal stumps and examination of teased osmic acid-fixed preparations. In no instance did strong stimulation cause any movement of the diaphragm, and no intact fibers were found in any of the material prepared for microscopic study.

*Discussion.* After finding that dogs with both phrenic nerves cut in the neck survive in excellent shape, it seemed possible that doing this operation by an intrathoracic route might in some way result in a different outcome. However, such is not the case, so the authors are left without an explanation of the recent report that dogs will not survive bilateral phrenectomy. On the contrary, it

seems that barring accidents incident to surgery this animal will always or nearly always survive. This conclusion is buttressed by the fact that half and even more of the intercostal nerves can be destroyed in addition without causing death.

*Summary.* Seven dogs were kept in good health and without respiratory distress for periods varying between 9 and 67 days after bilateral phrenectomy. All of the animals were eventually sacrificed, none died as a result of paralyzing the diaphragm. Electrical stimulation and microscopic examination proved that the phrenectomies were complete in all cases. It was established that following paralysis of the diaphragm at least half of the intercostal nerves, either upper or lower, may be sectioned without loss of the animal. The conclusion drawn is that reports or statements to the effect that the diaphragm is essential for life in otherwise intact grown dogs are erroneous.

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## Effect of Insulin on Maximal Rate of Renal Tubular Uptake of Glucose in Non-Diabetic Humans.* (20634)

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Although the various physiological effects of insulin are well known, the specific mode of action of the hormone has not been clearly delineated. At present, it is generally believed that insulin facilitates, in some manner, the phosphorylation of glucose which introduces carbohydrate into the metabolic processes of the cell. The exchange of glucose between glomerular filtrate and renal tubular cell appears to be a unidirectional process and is characterized by a maximal rate of tubular cell uptake of glucose. Glucose is completely removed from the filtrate until filtered load of glucose is increased to a point where the tubular resorptive mechanism is saturated. Further increments in filtered glucose appear in the urine. This situation would appear to provide a unique opportunity to study the effect of insulin on the rate of cellular uptake of glucose in the intact animal as contrasted to most *in vivo* work which has been concerned with the net exchange of glucose between cell and perfusing medium.

*Methods.* Twelve male subjects aged 47-68 years who were free of evidence of renal disease, cardiovascular disease, and derangement of carbohydrate metabolism were studied in the fasting state. The general procedure outlined by Goldring and Chasis(1) for the determination of Tm glucose was followed. After suitable priming dose, glucose was infused as a 30% solution in water at a rate of approximately 1.5 g per minute throughout the entire procedure. Inulin was added to the infusion fluid in quantities sufficient to maintain plasma levels of 20-25 mg %. Blood was sampled at the mid-point of each urine collection period through an in-

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