

receiving the same dosage died overnight. Further tests for such protection were, therefore, made.

*Animal Protection Experiments.* Eight white mice were injected intraabdominally with 0.5 cc of sterile broth Seitz filtrate obtained from a 24-hour culture. After 3 days, 1.5 cc of a similar filtrate from a broth culture grown for 96 hours, was likewise administered to these animals. Two days later all animals received intraabdominally 0.1 cc of a virulent *B. violaceus* broth culture. All animals survived and showed no evidence of illness during several weeks of observation.

Of 10 control mice, 5 received intraabdominally 0.5 cc and 5 received 1.0 cc of the same culture as used for the above animals and all died overnight.

Two control mice were injected with plain sterile broth in similar amounts, route and time periods as those receiving the broth culture filtrate. The injection 48 hours later of 0.1 cc of the living culture produced death within 24 hours.

It is concluded from these observations that this strain of *B. violaceus* isolated from an unique clinical case, is pathogenic for various laboratory animals. When the inoculum permits of survival for several days, the lesions produced are similar in the gross and microscopic aspect to those of the human.

It is further shown that the injection of *B. violaceus* broth culture filtrates into white mice affords protection to doses of the living microorganism that kill control animals overnight.

Preliminary experiments with the serum of mice injected with sterile filtrates suggest that antitoxic factors are developed by which passive protection may be transferred to normal animals.

## 11852

### A New Method for Regulated Vascular Obstruction.

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Restriction of blood flow in isolated vascular territories in living and unanesthetized animals has recently attracted attention. Some workers have fitted the blood vessels which are to be constricted into a type of rigid frame which reduces the diameter of the vessels.

Other investigators (Goldblatt,<sup>1</sup> Collins,<sup>2</sup> Rytand<sup>3</sup>) achieved the desired degree of ischemia by tightening loops of wire or thread around the vessel. Brotchner<sup>4</sup> and Blum, Schauer and Calef<sup>5</sup> employ a device which permits gradual mechanical closure of the vessel by means of 2 solid bars which are brought together by a screw to be actuated by a stiff wire from outside the animal.

The work of Mann, Herrick, Essex, and Baldes<sup>6</sup> correlated blood flow in vessels with a decrease in internal diameter brought about by local constriction. It was shown that a significant diminution of flow was achieved only after reduction of the internal diameter of arteries to a small percentage of the original.

The internal diameter of a vessel cannot very well be controlled quantitatively in the living animal because of the variability of the connective tissue reaction around the clamp among other reasons. Once a constricting loop or a rigid frame is applied to a vessel and the wound closed, the blood flow cannot be changed without further surgical intervention. Even then the percentage reduction of blood flow remains unknown until after the death of the animal, when model experiments can be performed on the flow of the blood in the constricted vessel.

The method which is described here attempts to regulate flow by employing the principle of the blood pressure cuff. This method should allow reversible and well defined obstruction of the vessels *in vivo*, when the pressure in the cuff can be controlled by a piece of rubber tubing which is brought out through the body wall.

Balloons of suitable dimensions have been obtained by the courtesy of Mr. H. E. Custer of the Dean Rubber Mfg. Co., 16th and Iron St., North Kansas City, Mo. If clamps of an irregular shape are needed it is relatively easy to make the balloons in the laboratory by dipping suitable molds into Latex\* and drying. The finished clamp consists of a frame made of Lucite or Bakelite, one or two balloons which are fitted in to this frame and connecting glass and rubber tubing. The assembled instrument may be sterilized by boiling. The solid part has a U-shaped cross section and accommodates the vessel to be restricted in the lower part of the groove. The flat

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<sup>1</sup> Goldblatt, H., Lynch, Hanzal and Summerville, *J. Exp. Med.*, 1934, **59**, 347.

<sup>2</sup> Collins, D. A., *Am. J. Physiol.*, 1936, **116**, 616.

<sup>3</sup> Rytand, D. A., *PROC. SOC. EXP. BIOL. AND MED.*, 1938, **38**, 10.

<sup>4</sup> Brotchner, R. J., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **40**, 264.

<sup>5</sup> Blum, L., Schauer, G., and Calef, B., *Am. Heart J.*, 1938, **16**, 159.

<sup>6</sup> Mann, F. C., Herrick, J. F., Essex, H. E., and Baldes, E. J., *Surgery*, 1938, **4**, 249.

\* Moulage No. 235, Vultex Chemical Co., 666 Main St., Cambridge, Mass.

balloon is then put on top of and parallel to the vessel, and the groove is closed by a tightly fitting cover. The cover is held in place by a ligature. The balloon is fixed to the cover by doubling back the free end and tying it to the frame. Stretching of the thin latex balloon is avoided by slipping its open end on a glass tube and connecting the tube to the frame of the clamp. Very sensitive vessels which should not come in contact with the solid part of the clamp may be constricted between two inflatable balloons. In order to obtain a reduction of blood flow which can be repeated consistently and quantitatively it is essential to observe a few precautions during the operation and application of the clamp.

The rubber balloon must be flaccid and without tension at atmospheric pressure. Both the artery and the balloon must fit tightly and snugly into the solid part. The balloon must fill the whole lumen of the U-groove without stretching of its walls. The artery must not be torn, constricted or kinked by the uninflated clamp. It is recommended to have available a series of clamps of varying cross-section in order to achieve the best results. Disregard of any of the above precautions may lead to results which are difficult to interpret.

Blood flow in the system composed of vessel and clamp is influenced by the following factors: pressure in the vessel, pressure in the constricting balloon, area of contact between cuff and vessel.

Of these factors the third has to be taken into consideration for the constriction of the clamp because the area of compression is determined by the length of the rigid part. If the area of contact is too small, pressures many times as high as the peak arterial pressures may fail to stop arterial flow completely. The cuff should compress the artery over a length of at least 8 mm in order to insure complete interruption of flow when the cuff pressure equals the peak arterial pressure.

The results reported below are taken from a femoral artery-femoral vein anastomosis in dogs. This arrangement eliminated peripheral resistance as a factor from our calibrations. For *in vivo* experiments the changes in peripheral resistance must be taken into account. Calibration over a range of pressure more extensive and continuous than reported can be undertaken in model circuits.

A Stolnikow-stromuhr had been interposed between artery and vein. The artery was constricted as described. The systolic and diastolic pressures were recorded optically by the "spoon" method (Sedgwick and Kubicek<sup>7</sup>). The manometer needle was inserted

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<sup>7</sup> Kubicek, W. G., Sedgwick, F., and Visscher, M. B., *The Review of Scientific Instruments*, in press.

TABLE I.

| Cuff pressure | Diastolic pressure | Systolic pressure | Flow<br>(in % of<br>unobstructed<br>120 cc/min) |
|---------------|--------------------|-------------------|-------------------------------------------------|
| 0             | 120                | 170               | 100                                             |
| 14            | 120                | 170               | 100                                             |
| 32            | 130                | 180               | 66                                              |
| 66            | 125                | 175               | 50                                              |
| 66            | 135                | 175               | 50                                              |
| 88            | 135                | 175               | 33                                              |
| 104           | 140                | 175               | 20                                              |
| 122           | 135                | 175               | 18                                              |
| 132           | 135                | 175               | 10                                              |
| 143           | 135                | 175               | 8                                               |
| 156           | 135                | 175               | 5                                               |
| 165           | 130                | 175               | 3                                               |
| 174           | 135                | 170               | 2                                               |
| 188           | 135                | 175               | 1                                               |
| 209           | 130                | 175               | 0                                               |
| 150           | 125                | 170               | 2                                               |
| 140           | 130                | 170               | 3                                               |
| 128           | 125                | 175               | 3                                               |
| 112           | 125                | 170               | 7                                               |
| 94            | 130                | 175               | 9                                               |
| 81            | 130                | 180               | 10                                              |
| 109           | 95                 | 140               | 5                                               |
| 85            | 95                 | 145               | 9                                               |
| 30            | 105                | 150               | 40                                              |
| 8             | 110                | 150               | 55                                              |
| 74            | 115                | 160               | 20                                              |
| 136           | 120                | 160               | 6                                               |
| 173           | 115                | 155               | 0                                               |
| 160           | 115                | 155               | 15                                              |
| 103           | 115                | 155               | 10                                              |
| 5             | 110                | 150               | 70                                              |

longitudinally into the artery, cardial to the constriction, with the bevel pointing towards the heart. The cuff pressure was read directly from a mercury manometer.

The reported figures indicate the gradations in flow which may be obtained with the above described device. It is believed that more satisfactory regulated and reversible restriction of blood flow can be achieved in this way than by other methods.