feedings for 6 days at a level of 100 mg per day, and by one preliminary chilling on the day prior to test (Table II). Adjuvant hardening was not required for the production of the thyroid effect. Discontinuance of the thyroid feedings was followed by a return toward the original sensitivity to gravitational effect within 5 to 7 days.

Summary. A technic is proposed through which changes in degree of tolerance for the upright position can be followed in the rabbit. Through use of the technic, a minimum tolerance is demonstrated in rabbits harboring depleting infections, and in rabbits softened by maintenance for a week or more in cages too small to permit exercise, and a heightened tolerance following subjection to either a preliminary chilling sequence, a single preliminary chilling followed by ascorbic acid administration, or a sequence of feedings of desiccated thyroid.

13888

Effect of Heparin on Retrograde Blood Flow.

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The use of heparin in the treatment of thrombosis is chiefly prophylactic.[1,2] There is some speculation that thrombi already formed are reduced in size by heparin, but no convincing evidence to that effect. It has also been suggested that heparin may increase the rate of blood flow, by reducing viscosity. If true, this fact would lend further reason for its use in the treatment of occluded vessels, by improving circulation through collaterals. This experiment is intended to show whether retrograde blood flow (*i.e.*, through a collateral circulation bed) is improved by administration of heparin.

Experiments. Dogs and cats were used. Under pentobarbital anesthesia, a femoral artery was ligated and severed. The amount of back-flow in 1-3 min from the distal end of the severed artery was then measured until constant readings were obtained, allowing sufficient time for opening of the femoral collateral circulation. Between readings, the free distal end was kept closed by a clamp, preventing excess loss of blood. Carotid pressure was recorded. Blood pressure was kept as constant as possible. A femoral vein was cannulated for infusions and for injection of heparin. After consistent control readings were obtained, the animals were given single doses of heparin (Liquæmin), the dose varying from 1000 to 3000 Howell cat units per kg. Readings were taken for one-half to 4 hr after heparinization—that is, until the clotting time had returned to normal or at least until the peak of heparinization was well passed.

The method was shown to be sensitive to changes in blood pressure, to blood dilution by saline infusion in excess of 100 cc and to excessive blood loss. Infusion of 200-300 cc of saline in dogs consistently produced a change in the rate of flow, varying from 15% to 30% increase—obviously a factor of dilution and consequent alteration of viscosity. This procedure was utilized at the conclusion of each experiment to check the reliability of the method.

Observations. Experiments were completed on 11 animals, including 9 dogs and 2 cats. In 7 of the 11 experiments, there was no increase of flow after heparinization; in 4 there was an increase of 15% to 30%, attributable to changes in the control conditions.

Conclusion. From the data obtained, hep-

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2 Murray, G., *Surg., Gynec., and Obstet.*, 1941, **72**, 340.

arin has no influence on the rate of retrograde blood flow from the distal end of an occluded artery. Heparin is probably of value in arterial occlusion, not by increasing the rate of flow, but by inhibiting the growth of the thrombus at the site of the occlusion.

13889 P

Endocrine Function of Ovarian Tissue after Growth or Storage *in vitro*.*

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In the course of studies on the effects of anterior pituitary hormones on ovarian tissue grown or stored *in vitro*, it was deemed necessary to develop a technic for determining whether the ability of such tissue to produce estrogen was lost. Accordingly a method was developed for testing the function of ovarian tissue by transplanting it, subsequent to storage or culture, into castrate female rats. The viability of ovarian tissues subjected to a variety of physical factors has been measured by Lipschütz[1] in terms of the hyperfeminization of male guinea pigs after implantation of the tissues on the surface of the kidney. Grafts of thyroid,[2] parathyroid,[2] pancreas,[3] pituitary,[4] and adrenal[5] tissues have been grown in culture prior to implantation in an attempt to acclimatize the tissue to its prospective host, but the routine implantation of cultured endocrine tissue to measure its function has not been utilized previously.

Ovaries from Sprague-Dawley rats, 28 to 32 days of age, were cut into pieces approximately one mm square, maintained *in vitro* under varied conditions, and implanted into the anterior chamber of the eye of adult, castrate females of the Sprague-Dawley strain. Vaginal smears were taken daily and the condition of the implant observed. A smear composed of cornified cells and a few leucocytes was considered a sign of the functional activity of the graft. The possibility of hypertrophied ovarian rests being the source of the vaginal stimulation was checked by removal of the grafts. In every case this procedure was followed by return to a typical castrate vaginal smear.

The ovaries were treated in the following way: (a) cut in human placental serum and implanted directly; (b) maintained *in vitro* for 5 days at 37°C, using the roller tube method of culture;[6] (c) prepared as in (b) but kept at 10°C for 5 days; (d) cut into small pieces, frozen in liquid air, and transplanted immediately or after storage for 2 days at —10°C.

A thick capsule of fibroblasts was observed in the 36-hr-old cultures maintained at 37°C. After implantation vascularization of the grafts by the small vessels of the iris occurred in 2 or 3 days. Rapid growth followed the establishment of a blood supply and in 10 to 14 days the grafts completely filled the anterior chamber. Large follicles and corpora lutea were seen in many of the grafts.

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