of immature rats appears to differ from that of sheep pituitary powder in that horse pituitaries seem to produce a greater follicular stimulation as compared to luteinization than those of similar preparations from sheep. This difference is especially noticeable in the case of castrate male horses. Pituitaries from 6 castrate males were prepared in powder form as described above. When this material was injected in saline emulsion in doses varying from 3 to 25 mg. the ovaries of the test rats showed predominantly follicular development, even in cases where the ovaries had increased in size as much as 210 mg. Slight luteinization was noticeable in only 2 cases out of 16 rats so injected. It seems therefore, that the pituitaries of these 6 castrate horses contained primarily follicular stimulating activity with very little luteinizing potentiality. This is in sharp contrast to pituitary powder from sheep which when injected in saline emulsion, in doses sufficient to produce ovaries of similar size, stimulated luteinization in every case.

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A Technique for Rendering Tissues Blood-free.

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The problem of rendering the tissues of a small laboratory animal like the rat, blood-free, must be solved if this animal is used in the study of tissue iron metabolism. In order that a more nearly correct iron content of a tissue may be obtained it will not suffice merely to correct for the iron contained in the blood of such a tissue, nor will the bleeding of the animal through decapitation or through severing a large artery render the tissues blood-free. The solution of this problem, we believe, rests in the development of a perfusion technique whereby the blood may be washed out of the living animal. A method adapted from Whipple's[1] procedure we wish to report here.

The method requires the services of 2 individuals. The equipment includes an ordinary dissecting kit, a ligature needle, a No. 23 hypodermic needle attached by means of a piece of rubber tubing

[1] Whipple, G. H., *Am. J. Physiol.*, 1926, **76**, 693.

to a 50 cc. burette equipped with pinch-cock, an operating board, and ligatures.

The animal is anesthetized with ether and fastened to an operating board, ventral side up. The skin is removed from the neck region extending from the chin, to and beyond the clavicles, and on either side of the median line to the axillae. The external jugular veins are dissected and linen ligatures are placed under the main branches of both veins. The internal carotid arteries are next dissected and one is prepared according to the method of Bethke, *et al.*[2] The latter carotid is, however, not severed to permit the flow of blood until later. The right external jugular vein is now closed to increase the blood flow through the opposite vein by means of an artery clamp. Into the latter vein now supported by ligatures and drawn slightly taut the hypodermic needle is inserted, as far forward anteriorly as possible with the point of the needle towards the heart. A flow of perfusion fluid into the vein assured (Locke's Solution) the needle is fastened in the vein with a ligature. The artery clamp on the opposite vein is removed. The operating board is tilted to an angle of 45° or more, the head of the animal lowermost. While a fine cut is being made in the specially prepared carotid artery, perfusion fluid is allowed to enter the animal. The inflow of the perfusion fluid should compensate as nearly as possible for the outflow of blood in order to maintain an adequate volume of fluid on which the heart may act. After 10 to 11 cc. have entered the animal the branches of the left jugular vein anterior to the point of insertion of the needle are severed to relieve the head of pressure, and after 19 to 20 cc. have entered, the same branches of the opposite vein are severed. These latter operations facilitate the thorough bleeding of the head. After a further introduction of 4 or 5 cc. the remaining intact internal carotid artery is severed and with the introduction of a total of 24 to 25 cc. of perfusion fluid the effluent from the severed carotids is clear and the perfusion is said to be complete.

The various tissues are removed, cut open and placed in saline to remove any superficial blood, and are then allowed to drain on clean cotton gauze and weighed to determine their fresh weight. They are then dried in a constant temperature oven at 100° for 48 hours to determine their moisture content. These tissues after having been ground in porcelain mortars are now ready for chemical analysis.

[2] Bethke, R. M., Steenbock, H., and Nelson, Mariana T., *J. Biol. Chem.*, 1923, **58**, 71.

In the fresh state, following a perfusion as described, the brain and the lungs are milky white in color, the spleen a pinkish red, the liver a deep buff or brown, the kidneys a paler buff color, while the heart is more pale and the muscles have their usual color. The fluid in the dorsal aorta is pinkish in color. The moisture content of these tissues does not indicate that edema has taken place.

Generally speaking, the use of the technique described has made for lower values of iron in tissues. Below are given the different iron contents of the various tissues of rats which have been prepared in different ways. In one instance the animals were killed with ether, in a second the animals were bled from the carotid and in a third according to the new technique. All tissues were removed from the animals in the same manner, placed in saline, and then allowed to drain on clean cotton gauze. The further preparation for analysis was the same in all cases. The iron determination was carried out according to Elvehjem's[3] modification of Kennedy's[4] method. Ashing time and temperature were maintained at a minimum. The samples used in each case were the composites of 11 animals of similar weight, age, sex, and from the same litters. Their average age was 10 weeks and they had been fed the stock ration *ad libitum*. The iron content of this ration averages about 253 mg. per kilo of dry ration.

TABLE I.
Iron Content of Rat Tissues Prepared in Various Ways Expressed on a Dry Weight Basis.

Tissue	Perfused mg. per 100 gm.	Bled mg. per 100 gm.	Not Bled mg. per 100 gm.
Bone	6.95	7.65	8.26
Brain	9.64	10.90	11.50
Heart	32.50	38.00	40.60
Kidney	26.20	26.00	43.50
Liver	60.40	56.60	81.60
Lung	23.00	35.50	64.40
Muscle	10.60	8.75	10.05
Spleen	84.20	93.00	108.50
Testicle	19.00	19.00	19.60

As more refined methods for the determination of iron in small amounts become available we feel that these differences will be amplified.

[3] Elvehjem, C. A., *J. Biol. Chem.*, 1930, **86**, 463.
[4] Kennedy, R. P., *J. Biol. Chem.*, 1927, **74**, 385.