*Summary.* Daily subcutaneous injections of 20 mg of yohimbine-HCl per kg of body weight for 26 to 33 days caused pseudopregnancy, increased ovarian and adrenal weights, and produced lobulo-alveolar development in female rats. The corpora lutea formed during this treatment were active as shown by their ability to produce decidual reactions in traumatized uteri. Induction of pseudopregnancy by single injections of 20 mg of yohimbine-HCl per kg of body weight was successful when given on the day of estrus. Daily injections of yohimbine had no effect on mammary development in male rats.

1. Rumry, F. L., *J. Pharmacol. and Exp. Therap.*, 1918, v11, 172.
2. Ludwig, F., Ries, J. V., *Arch. F. Gynak.*, 1931, v144, 280.
3. D'Amour, M. C., *Endocrinology*, 1934, v18, 235.
4. Fugo, N. W., Gross, E. G., *ibid.*, 1942, v31, 529.
5. Sulman, F., Black, R., *ibid.*, 1945, v36, 70.
6. Mares, S. E., Casida, L. E., *ibid.*, 1963, v72, 78.

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## Sodium and Potassium Content of a Small Artery.* (28509)

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Recently(1) we reported work in which we interposed a small open reservoir and constant volume pump between the arterial inflow and venous outflow of the rat tail and monitored ($Na^+$) and ($K^+$)† in the circulating medium with glass electrodes. When a sucrose-substituted, low $Na^+$ solution was perfused, resistance in the vascular bed declined quickly while a considerable amount of $Na^+$, apparently from cells, was added to the perfusing medium in this same time. These results led us to expect that the smooth muscle of the peripheral vascular tree would contain a large pool of $Na^+$. We now present substantiating data obtained directly by chemical analysis of the ventral tail artery, a small (500 $\mu$), predominantly muscular vessel.

Large (400 g) male albino rats under pentobarbitone-phenobarbitone anesthesia(2) were used. In one group, the ventral tail artery and a companion vein were cannulated and the tail then perfused at 0.65 ml/min and 37°C. The venous effluent was discarded. The system was allowed to run freely for 3 minutes using a basal solution of the following composition in mM/1: NaCl, 115.0; KCl, 5.0; $NAHCO_3$, 25.0; $NaH_2PO_4$, 1.2; $CaCl_2$, 2.1; $MgSO_4$, 1.2; dextrose, 11.0; calcium disodium versenate, 0.026. To this was added 4% Plasdone (polyvinyl pyrrolidine) as plasma colloid substitute. The base of the tail was then ligated and the perfusate changed for one modified by the addition of 400 mg% inulin (about 8 mM). This was then perfused without interruption for 30 minutes, the time necessary for inulin equilibration in this system.

In a second group, the renal pedicles were ligated and a carefully measured amount of inulin was injected into the tail vein and allowed to equilibrate for 2 hours. At the end of the procedure in both groups, a length of about 13 cm of tail artery was excised in less than 2 minutes (by stopwatch), then quickly dissected free of adventitia and placed in a pre-weighed stoppered bottle for immediate weighing. Inulin, Na and K in the tissue, in the venous effluent collected in the terminal minute of perfusion, or in an arterial sample were analyzed by conventional methods(2) modified for the small samples available here. The findings are

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† Na or K = sodium or potassium, not necessarily ionized $Na^+$ or $K^+$ = ions;
() = activity of the ions relative to the calibrating solution; [] = concentration relative to water.

TABLE I. Amount and Distribution of Water, Na, and K in Rat Tail Artery Compared with Typical Gastrocnemius Analysis.

| | Tail artery *in situ* perfusion | Tail artery *in vivo* circulation | Gastrocnemius *in vivo* circulation |
|---|---|---|---|
| No. of animals | 10 | 8 | 10 |
| Plasma or medium | | | |
| [Na], meq/l | 143 ± .7 | 143 ± .3 | |
| [K], meq/l | 4.57 ± .10 | 4.19 ± .12 | |
| Tissue (fat free wet) | | | |
| Total $H_2O$, ml/kg | 689 ± 5 | 657 ± 5 | 750 ± 1 |
| E.C.F. (inulin), ml/kg | 362 ± 14 | 374 ± 14 | 75.5 ± 3.0 |
| Total Na, meq/kg | 107.2 ± 1.7 | 98.4 ± 1.1 | 18.2 ± .3 |
| $Na_i$, meq/kg | 57.5 ± 3.4 | 47.1 ± 1.4 | 7.4 ± .3 |
| $[Na]_i$, meq/l | 176 ± 7 | 168 ± 5 | 11.0 ± .4 |
| Total K, meq/kg | 71.1 ± 2.5 | 58.4 ± 1.3 | 112.7 ± 1.0 |
| $K_i$, meq/kg | 69.5 ± 2.5 | 56.9 ± 1.3 | 112.3 ± 1.0 |
| $[K]_i$, meq/l | 216 ± 13 | 204 ± 9 | 167 ± 2 |

± = standard error.

shown in Table I together with a set of typical gastrocnemius values for comparison.

The partition of water (v), defined by the inulin space, is characterized by an almost equal distribution between cellular and extracellular phases, $v_i$ and $v_o$. The inulin estimate of the extracellular space, about 36% of total weight, seems quite reasonable. Not only was the same value obtained by the two entirely different procedures used here, but it is almost identical with that obtained in studies of taenia coli *in vitro* (3).

The total Na content of the rat tail artery is strikingly high, more than 5-fold that of gastrocnemius, for example. On the basis of the inulin space estimate for $v_o$, vascular smooth muscle cells also contain an amount of Na more than 5 times that of gastrocnemius. Indeed, calculations using any reasonable value for $v_o$ lead to the conclusion that these cells are obviously sodium-rich. The concentration of cell Na in relation to cell water, $[Na]_i$, is approximately equal to or a little greater than $[Na]_o$ (plasma or medium) regardless of the estimate of $v_o$. Thus, using the conventional equation for a 2-compartment system, i and o:

where m = total Na
v = total water
c = concentration of Na (relative to water),

$$\text{then } c_i = \frac{m - c_o v_o}{v - v_o}$$

$$\text{and } \frac{c_i}{c_o} = \frac{\frac{m}{c_o v} - \frac{v_o}{v}}{1 - \frac{v_o}{v}}$$

Clearly, $c_i = c_o$ at all values of $v_o$ when $m/c_o v$ is equal to 1. Further, where this ratio is close to unity, as it is here, a considerable inaccuracy in the estimate for $v_o$ can be tolerated without seriously changing the estimate for $c_i$. Accordingly, the tabulated value for $[Na]_i$ cannot be explained away by challenging the inulin estimate. Osmotic considerations alone, however, preclude so high a value for the concentration of mobile Na ions, $[Na^+]_i$. We must, therefore, conclude that ion binding plays a special and quantitatively important role in these cells.

The total K content of the rat tail artery is significantly lower than that of skeletal muscle but is not lowered to the same degree that total Na is increased. In cells, however, the sum of Na and K is of the same order of magnitude in artery and in gastrocnemius. The concentration of cell K relative to water, $[K]_i$, is higher than in skeletal muscle and the possibility of an important degree of binding must be seriously considered here too.

In general, the content and distribution of water, Na, and K observed here in a small muscular artery is very close to recently reported measurements in taenia coli *in vitro* (3). The artery is characteristically very

rich in Na and although much of this is in cells only a portion is ionized and, to an unknown but important degree, active.

*Summary.* The rat tail artery contains more than 5 times as much Na and about half as much K as gastrocnemius muscle. Inulin distribution indicates that cellular and extracellular water phases are about equal in magnitude. Calculation using the inulin space or any other reasonable estimate of water partition shows that much of the Na burden is necessarily cellular and non-ionized.

1. Friedman, S. M., Nakashima, M., Friedman, C. L., *Circulation Res.* in press.

2. Friedman S. M., Sréter, F. A., Friedman, C. L., *Gerontologia,* 1963, v7, 44.

3. Gocdford, P. J., Hermansen, K., *J. Physiol.,* 1961, v158, 426.

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## Some Observations on Lipid Metabolism and Adipose Tissue in the Vitamin E-Deficient Rat.* (28510)

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It is well known that vitamin E-deficiency coupled with a large intake of long chain poly-unsaturated fatty acids results in the development of yellow pigmentation of the body fat. This gross change is accompanied by striking histological changes consisting of the deposition of acid-fast and PAS-positive material within fat cells(1-3). Biochemical studies of this altered fat have revealed high levels of lipo-peroxides and increased levels of nitrogen, leading to the proposal that the lipids have undergone auto-oxidation, polymerization and coupling with proteins(4). The present studies were undertaken to define further some of the biochemical and metabolic changes associated with the development of this abnormality in the adipose tissue.

*Materials and methods.* A group of 18 rats weaned at 3 weeks of age were transferred to a Vit.E-deficient diet in which 30% of the calories were supplied as the free fatty acids of linseed oil(5). Controls consisted of weanlings and of adults receiving the E-deficient diet supplemented with 25 mg of α-tocopheryl acetate twice weekly. All animals were given oral supplements of Vit. A and D. Animals on the deficient diet were sacrificed in pairs at intervals up to 15 weeks. Samples of pararenal adipose tissue were removed and evaluated grossly. Lipids were extracted from the adipose tissue with chloroform:methanol and chloroform, and the solvents then evaporated with nitrogen. After further drying in a vacuum desiccator, the lipids were weighed on an analytical balance. Chromatograms of both the phosphatides and non-phosphatides were made on paper impregnated with silicic acid. After staining with Rhodamine 6G the lipid spots were detected under ultraviolet light(6).

Silicic acid column chromatography was used for quantitative separation of the lipid fractions into 4 groups(7). An additional group of non-phosphatides which has not been completely characterized was eluted from the column with 50 ml of ethyl ether immediately after the normal non-phosphatides.

To obtain further information concerning phospholipid metabolism in adipose tissue, orthophosphate labeled with P-32 was injected subcutaneously into 2 Vit. E-deficient and 2 control animals using a dose of one microcurie per gram of body weight. Animals were killed 24 hours later. Total lipids were extracted as above from liver and adipose

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