trophy increased. This means that the absolute amount of creatine in such hypertrophied hearts is definitely increased.

In those digitalis control animals who received 0.1 cat unit twice weekly, injected as Digalen or Digifoline, there was a distinct increase in the cardiac creatine content. Those that received larger doses, namely 0.3 or 0.5 cat unit, showed no relative elevation.

In one group of digitalized hearts there was found a definite but small lesion, the grade of cardiac hypertrophy being doubtful, however, when judged by the ratio of heart weight to body weight. These hearts show markedly elevated creatine values. In the digitalized hearts with distinct hypertrophy, the degree depending on the size of the experimental lesion, there is on the average slightly higher creatine percentage than normal, demonstrating again the fact that there is an absolute increase in the amount of creatine in these hypertrophied hearts.

8822 C

Studies in Histochemistry. VIII. Relationship Between Concentration of Vitamin C and Development of Pineal Gland.

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Although there have been many investigations of the occurrence of vitamin C in animal tissues in recent years, there have been no studies, as far the authors have been able to ascertain, of the vitamin C in the pineal gland. This probably is due to the fact that the gland is very small and usually difficult to obtain. In the fully developed cow it weighs but a few hundred mg.

It should be of interest to compare the vitamin C content of the pineal with that of other nervous tissues or organs of internal secretion such as the adrenal medulla, pars nervosa of the hypophysis, and brain, all of which are somewhat related embryologically. It should also be desirable to determine the concentration of vitamin C in the pineal gland in relation to its various stages of development from fetus to adult. The presentation of these determinations and comparisons is the purpose of the present investigation.

Using the Linderstrøm-Lang, Holter microtitration apparatus,[1,2]

and the micromethod for the estimation of vitamin C with 2,6-dichlorophenol indophenol[3], it was found that many determinations might be made on each gland with sufficient material left over for histological study as well.

The advantage of employing bovine tissue for this investigation depends (as in the case of the other organs studied) upon histological similarity to the human gland, availability in the fresh state, and, particularly in this case, convenient size As usual the glands were stored at —5°, and used for titration within one day.

When possible, a cylinder of tissue was cut out of the widest part of the pineal with a cork borer having a diameter of 4.2 mm., and the tissue was mounted on a freezing microtone, sections 30 μ thick were made, and every 5th section taken for titration. Because of the thinness of the gland, usually only 5 titration sections could be obtained in this manner, but the tissue was so homogenous that the titration values of the sections from any one pineal did not vary more than 0.20 c.mm. The accuracy fell with the lower titration values, but the low concentration of the vitamin in this gland makes lowered accuracy inevitable.

However, the pineals from fetuses, calves, and some of the cows were too small to permit use of the cork borer for sampling. In these cases the whole gland was frozen on the head of the microtone, and tissue was trimmed away with a scalpel so as to leave a block of tissue with a rectangular cross section on the microtome. The dimensions of this rectangle were determined with a vernier caliper so that the volume of each 30 μ section might be obtained. The smallest sections made in this manner were 4.5 x 3.1 mm., and the largest 8.0 x 4.8 mm. The tissue trimmings were saved for histological examination, as were the portions of tissue from the larger pineals left after removing the samples with the cork borer.

The material saved for histological examination was fixed in Bouin's fluid, embedded in paraffin, sectioned, stained with hematoxylin and eosin, and mounted.

The histological changes that occur in the development of the bovine pineal gland as demonstrated by the hematoxylin-eosin stain are briefly summarized: In the 40 cm. fetus (5.2 months old) the pineal is composed of small clusters or rosette-like arrangements of cells that have a uniform appearance. They are separated by a

[1] Linderstrøm-Lang, K., and Holter, H., in Nord, F. F., and Weidenhagen, R., *Ergebrisse der Enzymforschung*, Leipsig, 1934, **3**, 309.

[2] Glick, D., *J. Chem. Educ.*, 1935, **12**, 253.

[3] Glick, D., *J. Biol. Chem.*, 1935, **109**, 433.

TABLE I.
The Vitamin C Concentration in the Bovine Pineal at Different Stages of Development.

No.	L	C	I	D	E	M	N	O	A	B	K
Animal	40 cm. Fetus (5.2 mo.)	70 cm. Fetus (7.2 mo.)	80 cm. Fetus (8 mo.)	Calf	Calf	Calf	Calf	Calf	Cow	Cow	Cow
Aver. titration per section. c.mm. dye minus .60 c.mm. blank	.50	.80	.90	.53	.40	.45	.54	.52	.40	.37	.22
Vitamin C per section, micrograms	.06	.10	.11	.07	.05	.06	.07	.07	.05	.05	.03
Mg. vitamin C per gm. tissue	.15	.24	.27	.16	.12	.15	.16	.16	.12	.12	.07

scant fibrillar substance. In the 80 cm. fetus (8 months old) the cells have a more distinct rosette arrangement, but they are slightly larger; cells are often situated around the capillaries. In the calf the several types of cells are uniformly distributed between irregular bundles of fibrillar material and the rosette formations have disappeared. In the adult this variety of cell types is more clearly distinguished, and glial fibrils are present.

From Table I it may be seen that the vitamin C concentration increased from 0.15 mg. per gm. of tissue in the 5.2-months fetus to a maximum of 0.27 mg. per gm. in the 8-months fetus. The concentration then decreases through the calf to the adult stage, reaching its lowest level of 0.07-0.12 mg. per gm. in the older animals. Thus the general principle that the vitamin C concentration in organs falls with increasing age as demonstrated by King and coworkers,[4] von Euler and Klussmann,[5] and Plaut and Bülow,[6] applies to the pineal gland as well. The apparent exception to this rule in the case of the thymus, if only the glandular tissue is considered, has been pointed out in a preceding paper.[7]

If a comparison is made of the vitamin C concentrations of the brain[6,8] and pineal gland, it is found that they are approximately the same. These values however are much lower than those observed for the adrenal medulla[9] or pars nervosa of the hypophysis.[10] Hence there appears to be no relationship between the vitamin C concentration in nervous tissues in different parts of the body.

It is not known whether vitamin C is involved in the production or function of the hormone found in the pineal gland. But it may be considered probable that the vitamin is concerned with the respiration of the pineal tissue just as it seems to be concerned with the respiration of brain tissue.[8,11]

Summary. The concentration of vitamin C has been determined in the bovine pineal gland at various stages of development from the early fetus (just over 5 months old) to the adult. Histological examinations have been made at these various stages in an attempt to correlate the cellular state with the concentration of vitamin C present.

[4] Yavorsky, M., Almaden, P., and King, C. G., *J. Biol. Chem.*, 1934, **106**, 525.

[5] von Euler, H., and Klussmann, E., *Z. physiol. Chem.*, 1933, **217**, 167; 1933, **219**, 215.

[6] Plaut, F., and Bülow, M., *Z. Neurol.*, 1935, **152**, 84.

[7] Glick, D., and Biskind, G. R., *J. Biol. Chem.*, 1936, **114**, 1.

[8] Plaut, F., and Stern, K., *Naturwissenschaften*, 1935, **23**, 557.

[9] Glick, D., and Biskind, G. R., *J. Biol. Chem.*, 1935, **110**, 1.

[10] Glick, D., and Biskind, G. R., *J. Biol. Chem.*, 1935, **110**, 583.

[11] Malmberg, M., and von Euler, H., *Z. physiol. Chem.*, 1935, **235**, 97.

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8823 C

Effect of Administration of Desiccated Thyroid During Pregnancy in the Albino Rat.

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Several years ago the observation was made by one of us (S.L.) that pregnant rats which received desiccated thyroid did not show the rather marked reduction in weight noted in similarly treated animals which were not pregnant. This observation has been extended and the rate of oxygen consumption under comparable conditions has been determined.

Methods. Thirty-three young, mature rats of approximately the same weight (180 gm.) and age and from the same colony were used. These were divided into 3 groups of 10, 11, and 12 rats respectively. After a period of 5 days of control feeding of the same amount (15 gm. daily) of a standard diet (Steenbock), they were assumed to be in a normal, pre-experimental condition. All rats received the same diet and the amount given above. The only variables in the three tests were pregnancy and the feeding of desiccated thyroid.

The *first group* were *not mated* and received in their diet daily 100 mg. of *desiccated thyroid.* This was continued for a period of 16 days, during which time body weight and rate of O_2 consumption were determined at 2- to 3-day intervals. This group served as *thyroid-fed, non-pregnant controls,* a 16-day period being chosen because that was the period of treatment and observation in the thyroid-fed, pregnant animals.

The *second group were mated* and given the same diet *without thyroid.* Determinations of O_2 consumption and body-weight were started after mating and continued to delivery. This group served as *non-thyroid fed, pregnant controls.*

The *third group were mated* and given the same diet *with thyroid.* The thyroid was not given until from 5 to 10 days after placing