present at the same time an active pituitary gland. The supplying of adequate amounts of caloric material is insufficient of itself to enable the mammary gland to respond to estradiol. Either the change in internal metabolism brought about by the growth fraction is essential to mammary development or some specific mammogenic factor is needed.

On the basis of the work of Nathanson, *et al.*,[6] the pituitary factor will apparently only affect the mammae in the presence of estrogens. Since Turner's extracts have not been demonstrated to be estrogen-free, it may be that their effectiveness in hypophysectomized rats was due to the combined presence of small amounts of such substances and some pituitary factor as yet not clearly distinguished. This apparently was present in both Nathanson's and Turner's extracts.

The experiments of Astwood, *et al.*,[5] can best be explained by assuming that the pituitary gland is sensitive to undernutrition. This was first shown by Moore and Samuels[9] in connection with the gonadotropic factor. If this assumption is correct Astwood's rats failed to show mammary development because of lack of the pituitary factor.

Summary. Well nourished hypophysectomized rats did not show mammary development when stimulated by large doses of estradiol benzoate for a period of 28 days. When this result is correlated with those of other workers it appears that both estrogens and some pituitary factor must act directly on the mammary gland to produce normal development.

11999 P

Efficiency of Electrical Energy Production by Surviving Frog Skin, Measured by Iodine Coulometer.*

PAUL STAPP. (Introduced by Ancel Keys.)

From the Laboratory of Physiological Hygiene, University of Minnesota, Minneapolis, Minn.

Surviving frog skin is the seat of an electrical potential which has been related to factors affecting its metabolic activity.[1,2] To deter-

9 Moore, C. R., and Samuels, L. T., *Am. J. Physiol.*, 1931, **96**, 278.

* Based on data submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the University of Texas, Austin, Texas.

1 Lund, E. J., *J. Exp. Zool.*, 1926, **44**, 383; 1928, **51**, 291, 327; 1931, **60**, 249.

2 Francis, W. L., *Nature*, 1933, **131**, 805.

mine the efficiency of electrical energy production through an external circuit by surviving frog skin it is necessary to measure the joules of electrical energy and the oxygen consumption of the skin during the same interval of time. An iodine coulometer[3] of low resistance (18-22 ohms) with sufficient electrode area to minimize polarization within the range of skin potentials, was employed to integrate the quantity of current passing through the external circuit. The coulometer was calibrated against known, constant current densities for measured intervals with a microammeter in series as a check. Average voltage was determined with a standard potentiometer by readings at 5 to 10 minute intervals.

The oxygen consumption was found by difference between initial and final oxygen concentrations in 25 ml samples of the Ringer's solution surrounding the skin, using the Winkler method for analysis.

Experiments were carried out in duplicate on symmetrical pieces of frog skin *(Rana catesbiana)* of 24 cm^2 area. Each piece of skin was clamped between a pair of iso-electric cup electrodes with 60 ml of Ringer's solution on each side. The electrodes were of lead amalgam-lead chloride covered by a surface layer of saline agar. The external circuit consisted of Ringer's solution, the electrodes, a coulometer and a microammeter in series. A potentiometer was connected across the electrode terminals.

Results of a typical one-hour determination at 25°C: The coulometer measured 1.497 coulombs. The average potential for 6 readings was 0.039 volts. The oxygen consumption was 0.239 ml at standard temperature and pressure. The respiratory quotient of the isolated skin was not determined. The highest and lowest respiratory quotients for the intact frog range from 0.94 to 0.72.[4] Calculations of the efficiency based on calorie equivalents for each of these respiratory quotients were found to differ relatively little. From the equation

$$\frac{EIT/4.181 \times 100}{\text{ml. } O_2 \times C} = \%\ \text{Efficiency}$$

Where E = average volts, IT = coulombs, 4.181 = factor converting joules to calories, and C = calorie equivalent for a given respiratory quotient. Substituting experimental values in this equation:

$$\frac{0.039 \times 1.497/4.181 \times 100}{0.239 \times 4.702} = 1.0\% \text{ for a R.Q. of 0.94.}$$

[3] Washburn, E. W., and Bates, S. J., *J. Am. Chem. Soc.*, 1912, **34**, 1341.

[4] Dolk, H. E., and Postma, N., *Z. f. Vergl. Physiol.*, 1927, **5**, 417.

The corresponding value for the R.Q. of 0.72 gave a value of 1.06%. The chemical efficiency was determined from the relation:

$$\frac{\text{Thiosulfate equivalent of coulombs} \times \text{volts} \times 100}{\text{Thiosulfate equivalent of oxygen}} = \text{Efficiency}$$

Substituting experimental values:

$$\frac{1.625 \times 0.039 \times 100}{4.49} = 1.23\%.$$

The control determination gave values of 0.93% for a 0.94 R.Q., 0.99% for a 0.72 R.Q., and 1.13% for the chemical efficiency. Average values for 20 experiments are shown in Table I.

TABLE I.

No. of Exps.	Duration	Avg volts/hr	Coulombs per hr	Gram calories per hr	Cc O_2/hr at standard T. and P.	Efficiency from R.Q. % 0.72	Efficiency from R.Q. % 0.94	Chemical Efficiency
14	2 hr	.0235	1.134	.0068	.130	1.10	1.04	1.20
3	1 hr	.0260	1.208	.0086	.140	1.37	1.30	1.58
3	30 min	.0346	1.671	.0107	.132	2.50	2.38	2.87

Other determinations made with external circuit resistances varying from 65 to 1500 ohms indicate that electrical energy output and efficiency diminish with rising resistance. If we assume that the external circuit resistance of 65 ohms is approximately equal to the internal resistance of the skins in the 30-minute determinations (Table I), the total electrical energy output of the skin would be in the neighborhood of 5 or 6% of the total energy metabolism. A gradual fall of the energy production curve during the first 30 minutes, followed by a steeper decline during subsequent intervals accounts for the higher efficiency in the shorter determinations. Exposure to saturated air for 8 to 10 hours caused a 40 to 50% increase in energy production during the succeeding 1- to 2-hour determinations. That the decline in energy production was not necessarily related to a continuous external short circuit was shown by a similar decline in a skin whose external circuit was closed only for microammeter readings.

Further investigation of the effect of varying oxygen tension, temperature and previous state of nutrition on the energy output are being undertaken to elucidate the nature of the relation between metabolic processes and electrical energy output of the frog skin.