some time following the first operation—we believed the operation would be successful in a greater number of cases. In this paper we report that in 6 attempts at transplantations with this method all were successful.

All the rats used in the above experiments were virgin animals, 3 to 5 months old at the time of the initial operations, and all transplants were made during dioestrous. The transplant was successful in each case. All 6 rats were in excellent health and showed no insufficiency symptoms at the time of removal of the ovary containing the adrenal transplant 50 to a little over 60 days following removal of the second adrenal, *i. e.*, a much longer interval than the longest survival period of our successfully adrenalectomized rats;[2] all except one, in which accessory adrenal tissue was found, showed typical insufficiency symptoms and died 3 to 13 days after removal of the transplant. It is to be noted that the operation did not interfere with normal pregnancy, and that pregnancy did not prevent insufficiency symptoms and death when the transplant was removed.

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A New Method of Determining Solubilities Based on Stability of Phthalate Buffers of Low pH at Low Temperatures.

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The disconcerting finding that buffers of potassium acid phthalate-HCl become more alkaline on standing led to a consideration of the cause of this change and to the discovery of a new method of determining solubilities.

The buffers were made according to the directions given by Clark.[1] The immediate determination of the pH of these buffers by the quinhydrone electrode gave values close to those expected, but subsequent pH determinations showed that a change had taken place on standing. This was particularly true at 13°C., whereas at 20°C. the changes were not great. A deposition of crystals could be observed accompanying the change in the pH of the buffers and this crystallization seemed to be proportional to the degree of change in the pH values. After the effect had been noticed in one set of buffers a new series was made up and the pH values determined by

[1] Clark, W. M., Determination of Hydrogen Ions. 1928.

the quinhydrone electrode within 2 hours after making it up. Each buffer in the series was then divided into 2 pyrex flasks. One set was placed at 20°C. and the other at 13°C. for 48 hours after which the pH values were again determined. The results are shown in Table I.

TABLE I.
Experiments at 20°C.

Determined pH		$C_H+ \times 10^{-2}$	Molar Concentration. Undissociated Phthalic Acid.		
2 Hours A	48 Hours B	Change A—B	2 Hours C	48 Hours D	Change C—D
2.26	2.33*	—.081	.041	.040	—.001
2.44	2.50*	—.047	.038	.036	—.002
2.64	2.66	—.010	.033	.032	—.001
2.74	2.70	+.018	.030	.031	+.001
2.80	2.79	+.004	.028	.029	+.001
3.97	3.97	.000			
Experiments at 13°C.					
2.26	2.49*	—.225	.041	.037	—.004
2.44	2.64*	—.134	.038	.033	—.005
2.64	2.73*	—.043	.033	.031	—.002
2.74	2.79	—.020	.030	.029	—.001
2.80	2.89	—.029	.029	.026	—.003
3.97	3.97	.000			

* Crystals.

The results show that the pH changed appreciably at 20°C. in buffers of pH 2.26 and 2.44 but not in the buffers of higher pH value. At 13°C. the pH changed considerably more in each of the above mentioned buffers as well as changing definitely in the buffers up to pH 2.80. The exact change in C_H+ after 48 hours is shown in column 3 of the table. The buffers showing crystal formation are indicated in the table by the asterisk (*) and seem to coincide with high values for change in C_H+.

The changes in the pH values noted above are to be expected from the solubility of phthalic acid as given by Seidell.[2] The solubility of phthalic acid as determined by the content of a saturated solution is approximately 6.2 gm. per liter at 20°C. and 5.2 gm. per liter at 13°C. The solubility of the salt of phthalic acid, however, is very high. The C_H+ of the pure acid may be given approximately by the following equation:

[2] Seidell, A., Solubility of Inorganic and Organic Compounds, 1919, I, 490; 1928, II, 1347.

(1) $C_{H^+} = \sqrt{K_1 \cdot \text{Conc. of Acid.}}$

At 13°C. the total concentration of the undissociated acid will be $1-\alpha$ and assuming $pK_1 = 2.94$ at 13°C. and 2.93 at 20°C. we have

$$(2)\quad \text{pH } 13°\text{C.}-pK_1 13°\text{C.} = \log\frac{\alpha}{1-\alpha};\ 2.22-2.94 = \log\frac{\alpha}{1-\alpha},\ 1-\alpha = 84\%.$$

$$\text{Similarly at } 20°\text{C.};\ \ldots\ldots 2.18-2.93 = \log\frac{\alpha}{1-\alpha},\ 1-\alpha = 85\%.$$

The undissociated acid of a saturated solution of phthalic acid would be 0.026 Molar at 13°C. and 0.031 Molar at 20°C. In the M/20 acid phthalate buffers used above, the concentration of undissociated phthalic acid may be calculated from the pH and total concentration of phthalate by using equation (2). The values obtained are shown in the fourth and fifth columns of the table. In the experiments at 20°C. there is a definite tendency for the phthalic acid to crystallize out until a final saturation value of 0.031 Molar is reached, whereas at 13°C. the process is more extensive and approaches the saturation value of 0.026 Molar. That a final end to this process had not come in the 48 hours is evident from the fact that buffers of pH 2.2 and 2.5 changed to pH 2.46 and 2.76 respectively on standing several weeks at 13°C.

This paper points out (1) that M/20 acid phthalate buffers of pH values less than 2.7 are supersaturated with phthalic acid at 20°C. and buffers of pH values less than 2.9 are supersaturated at 13°C. Consequently these buffers do not have a stable pH value and may be a source of considerable error where work is carried on at temperatures below 25°C. As this buffer system is extensively employed in biological work the above dangers are noteworthy.

(2) The solubility of the weak acid or the weak base employed in buffers as affected by changes in temperature and concentration may be accurately determined by a study of the accompanying change in the pH values; thus, a new method of solubility determination is here indicated.