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Chemical Concentration of Mammogen from Prehypophyseal Tissue.*

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With the development of an assay technic for the duct-growth entity of mammogen,[1] our attention has turned to the study of methods of extraction and purification. In previous reports from this laboratory,[2, 3] a method has been described for the extraction of the lactogenic and carbohydrate-metabolism hormones. In order to determine whether this method would be equally satisfactory for the extraction of mammogen, the following procedure was carried out.

Pituitary tissue from pregnant cattle was dried with acetone and ether. Assay showed there had occurred a loss of 60% of mammogen. The dried tissue was then extracted with 60% alcohol at a pH of 9 to 10 and the *initial extract* precipitated by adjusting the pH to 5.7 and increasing the alcohol content to 86%. As the *initial extract* contained only 5% as much activity as the fresh tissue, it was clear that this method was unsatisfactory. Further, the residual tissue was found to contain slightly more mammogen per unit than did the initial extract.

In a second experiment, the fresh pituitary tissue was not dried, but immediately extracted with alcohol and the *initial extract* precipitated as described above. The 86% alcohol solution was then vacuum distilled. The oily residue contained a mouse unit per 3 mg. It was estimated that about 90% of the mammogen present in the fresh tissue was recovered.

As the mammogenic hormone appeared to be soluble in the lipid solvents, several methods were tried. Extraction with 95% alcohol for 2 days, followed by vacuum distillation produced a residue containing slightly less than 1 mouse unit in 40 mg. Extraction with several volumes of hot ether-alcohol (1:3), resulted in a preparation containing 1 unit per 3 to 4 mg which was equivalent to about 100% of the potency of the fresh tissue.

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1 Lewis, A. A., Turner, C. W., and Gomez, E. T., *Endocrinology*, 1938, **23**, 672.

2 Bergman, A. J., and Turner, C. W., *J. Biol. Chem.*, 1937, **118**, 247.

3 Bergman, A. J., and Turner, C. W., *J. Biol. Chem.*, 1938, **123**, 471.

TABLE I.
Potency of Mammogen in Extracts of Prehypophyseal Tissue from Pregnant Cattle.

Extract	No. of mice	Days injected	Total dose, mg	No. of mice showing mammary stimulation	No. of mice showing no mammary stimulation
Fresh prehypophysis from pregnant cattle	53	6	105	30	27
Acetone and ether dried prehypophysis	8	14	28	0	8
	12	6	40	5	7
Initial extract	4	21	21	0	4
	12	6	120	6	6
Inert material (for most pituitary hormones)	3	4	40	0	3
	3	4	80	1	2
	10	6	96	5	5
Residue—86% alcohol distillation; second extraction	12	6	1	3	9
	11	6	3	5	6
Residue—95% alcohol distillation; cold extraction	5	6	20	1	4
	8	6	40	3	5
Residue—95% alcohol distillation and ether-hot extraction	10	6	3	3 1?	6
	11	6	4	7	4

Summary. Acetone and ether drying of prehypophyseal tissue from pregnant cattle was observed to result in a 60% loss of mammogenic hormone. Similarly mammogen was not precipitated to any extent from 86% alcohol at pH 5.7. The residues obtained from the vacuum distillation of 86% alcohol and from hot alcohol-ether extraction were potent in mammogen.

These observations indicate that mammogen has distinctly different chemical properties from the lactogenic and carbohydrate-metabolism hormones which are isoelectrically precipitated in alcohol. Mammogen appears to be extracted in lipid solvents and remains in solution at high concentrations of alcohol. It can be recovered as an oily residue upon the vacuum distillation of the alcohol or ether-alcohol extracting solutions.