adrenocortical activity. The functions of adrenal cortex were normal in paranoia. In this disease the psychical disturbance is limited within certain spheres of mental organization and condition of stress is less in comparison with other psychotic diseases. The functional activity of the adrenal cortex, therefore, possibly did not change. Diminished urinary excretion of 17-ketosteroids in schizophrenia might be due to hypofunction of testes Leydig cells, of adrenal cortex or of both these organs. Hemphill *et al.*(18) observed degenerative changes in the testes of schizophrenics and changes in most patients were similar to those of the testes of hypophysectomized animals. Urinary excretions of 17-ketosteroids also decreased both after electric convulsion therapy(18,19) and prefrontal leucotomy (18). The disorder in schizophrenia seems likely to be in the cerebral control of pituitary.

Summary. Eosinopenic response to injected epinephrine was studied in patients suffering from schizophrenia, paranoia, depression and mania. Eosinopenic response was lower in cases of schizophrenia and mania, higher in depression and normal in paranoia. Eosinopenic response to injected ACTH which was studied in 8 schizophrenic patients was very low. 24-hour urinary excretion of 17-ketosteroids by these schizophrenic patients was also very low.

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1. Pincus, G., Hoagland, H., Freeman, H., Elmadjian, F., Romanoff, A. B., *Psychosom. Med.,* 1949, v11, 74.

2. Stein, M., Ronzoni, E., Gildea, E. F., *Am. J. Psychiat.,* 1951, v108, 450.

3. Benda, P. H., *Encephale,* 1951, v40, 228.

4. Faurbye, A., Vestergaard, P., Kobbernagel, P., Nielsen, A., *Acta. Endocrinol.,* 1951, v8, 215.

5. Seliger, H., *Ther. Umsch.,* 1954, v11, 54.

6. Binswanger, H., Meier, L., *Schweiz. Med. Wschr.,* 1953, v82, 513.

7. Pechstein, H., *Psychiat. Quart.,* 1952, v26, 425.

8. Reiss, M., Hemphill, R. E., Early, D. F., Maggs, R., Cook, E. R., Pelly, J. E., *J. Clin. Exp. Psychopath.,* 1953, v5, 236.

9. Heidrich, R., Hampel, R., *Psychiat. Neurol.,* 1953, v5, 236.

10. Parsons, E. H., Glidea, E. F., Ronzini, E., Hulbert, S. Z., *Am. J. Psychiat.,* 1949, v105, 573.

11. Henneman, P. H., Wexler, H., Westenhaver, M. M., *J. Lab. Clin. Med.,* 1949, v31, 107.

12. Davidson, R. A., Koets, P., Kuzell, W. C., *J. Clin. Endocrinol.,* 1947, v7, 202.

13. Das Gupta, S. K., Singh, H., *Indian J. Med. Res.,* 1956, v44, 31.

14. Thorn, G. W., *Trans. & Studies Coll. Physicians Phila.,* 1952, v19, 95.

15. Pincus, G., Dorfman, R. I., Romanoff, L. P., Rubin, B. L., Bloch, E., Carlo, J., Freeman, H., *Rec. Prog. Horm. Res.,* 1955, v11, 307.

16. Lehman, M. E., Turski, M., Cleghorn, R. A., *Canad. Med. Assn. J.,* 1950, v65, 325.

17. Vargha, M., Tass, Gy., Huszak, I., *Psychiat. Neurol. u. Med.,* 1954, v6, 29.

18. Hemphill, R. E., Taylor, A. L., *Recent Progress in Psychiatry,* 1943, 410.

19. Alexandre, H., Ferreira, A., *J. Brazil. Psiquit.,* 1951, v1, 203.

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Potentiating Effects of Reserpine on Thyrotoxicity in the Rat.* (24291)

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Considerable data are available indicating that reserpine under various experimental conditions has an anti-thyroid effect. Thus Kuschke and Gruner(1) reported that large doses of reserpine prevented the increase in oxygen consumption produced by parenterally administered thyroxine in rats. De Felice *et al.*(2) observed that reserpine reduced oxygen consumption of euthyroid and hyperthyroid guinea pigs. A direct *in vitro*

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antithyroid effect of reserpine consisting chiefly of inhibition of organic binding of I^{131} has been reported(3). Moncke(4) found that chronic reserpine therapy over a period of months produced "normalization" of basal metabolic rate in a group of 15 hyperthyroid patients. In contrast to the above Goodman *et al.*(5) found that reserpine at the dose levels employed clinically for treatment of hypertension did not affect thyroid function in man; and Ford *et al.*(6) were unable to demonstrate any change in basal metabolic rate or I^{131} uptake of the thyroid gland in euthyroid patients after chronic treatment with a total alkaloid extract of *Rauwolfia serpentina*. Since rats fed highly purified diets are particularly sensitive to thyroid feeding as evidenced by early death, apparently due to cardiac failure(7,8), it was felt that if reserpine has an anti-thyroid effect it might be manifest in terms of an increased survival time in the immature thyroid-fed rat. Findings indicate, however, that rather than prolonging survival as might have been anticipated, reserpine in large doses significantly increased the toxic effects of thyroid feeding.

Procedure. The basal ration consisted of sucrose, 65.8%; casein,† 24%; salt mixture,‡ 5%; corn oil 5%; and l-cystine, 0.2%. To each kg of the above diet were added the following vitamins: thiamine hydrochloride, 20 mg; riboflavin, 20 mg; pyridoxine hydrochloride, 20 mg; calcium pantothenate, 60 mg; nicotinic acid, 100 mg; ascorbic acid, 200 mg; biotin, 4 mg; folic acid, 10 mg; para-aminobenzoic acid, 400 mg; inositol, 800 mg; Vit. B_{12}, 150 μg; 2-methyl-naphthoquinone, 5 mg; choline chloride, 2 g; Vit. A, 5000 U.S.P. units; Vit. D_2, 500 U.S.P. units; and alpha-tocopherol acetate, 100 mg. The vitamins were added in place of an equal amount of sucrose. Male and female rats of the Holtzman strain were selected shortly after weaning and were fed either the basal ration indicated above or the basal ration plus the supplements listed in Table I. Food and water was provided *ad lib.* Animals were fed daily and all food not consumed 24 hours after feeding was discarded. Feeding was continued for 6 weeks or until death whichever occurred sooner. Initial body weight, number of animals per group and results obtained are summarized in Table I.

Results. In agreement with earlier findings(7,8) growth was retarded and rats failed to survive when fed a highly purified ration containing massive doses of thyroid. The effects obtained were proportional to amount of thyroid administered. Reserpine§ when fed with the basal ration at a level of 10 mg/kg of diet had no deleterious effects on either growth or survival; when fed at a level of 20 mg/kg diet, however, it resulted in significant growth retardation although no deaths occurred during the experimental period. Combined supplements of reserpine at a level of 20 mg/kg ration and desiccated thyroid at all levels caused 100% mortality within an experimental period of 10 days. Reserpine when fed at a level of 10 mg/kg ration in conjunction with the 0.5% thyroid supplement resulted in a weight increment significantly less than that obtained with the thyroid supplement alone and also decreased average length of survival. It is apparent from these findings that whereas decreasing doses of thyroid did not significantly affect the deleterious effects of combined reserpine-thyroid supplementation when reserpine was fed at a level of 20 mg/kg ration, decreasing the dosage of reserpine when thyroid was kept at the 0.5% level reduced the severity of the effects obtained. The potentiating effects of reserpine on symptoms of thyrotoxicity were observed both in male rats (Exp. 1) and female rats (Exp. 2).‖

† Vitamin-free Test Casein, General Biochemicals, Chagrin Falls, O.

‡ Hubbel, Mendel and Wakeman Salt Mixture, General Biochemicals, Chagrin Falls, O.

§ Reserpine was kindly provided by Dr. George Cronheim of Riker Labs., Los Angeles, Calif.

‖ Similar studies were conducted with female rats of the Holtzman strain fed a natural food stock ration (ground Rockland Rat Diet, Arcady Farms Milling Co., Chicago, Ill.). No deaths occurred in rats fed the unsupplemented stock ration, the stock ration plus 0.125% desiccated thyroid or the stock ration plus 20 mg reserpine/kg of diet (6 animals/group). All 6 rats fed the stock ration supplemented with above amounts of both thyroid and reserpine, however, died within 12 days.

TABLE I. Effects of Reserpine on Weight Increment and Survival Time of Thyroid-Fed Rats.*

Supplements fed with basal ration	Gain (g) in body wt after following days of feeding 21st	42nd	Mortality, %	Avg survival time of decedents, days
Exp. 1				
None	133.6 (10)	213.6 (10)	0	
.125% thyroid	118.4 (10)	193.9 (10)	0	
.25% "	103.0 (10)	134.2 (3)	70	35.2
.5% "	95.4 (10)	143.4 (1)	90	31.6
10 mg reserpine/kg diet	144.8 (10)	228.6 (10)	0	
20 mg *Idem*	94.2 (10)	170.5 (10)	0	
.125% thyroid & 20 mg reserpine/kg diet			100	8.7
.25% *Idem*			100	8.3
.5% "			100	6.3
.5% thyroid & 10 mg reserpine/kg diet	50.3 (5)		100	21.8
Exp. 2				
None	96.9 (10)	139.2 (10)	0	
.125% thyroid	98.2 (10)	141.2 (10)	0	
20 mg reserpine/kg diet	62.0 (10)	107.2 (10)	0	
.125% thyroid & 20 mg reserpine/kg diet			100	8.6
Exp. 3				
None	98.4 (6)	144.2 (6)	0	
.125% thyroid	98.5 (6)	146.7 (6)	0	
20 mg reserpine/kg diet	83.2 (6)	108.4 (6)	0	
.125% thyroid & 20 mg reserpine/kg diet			100	12.4
200 mg chlorpromazine HCl/kg diet	94.4 (6)	123.9 (6)	0	
.125% thyroid & 200 mg chlorpromazine HCl/kg diet	84.7 (6)	108.6 (6)	0	
2700 mg 2-methyl-2-n-propyl-1,3-propanediol dicarbamate/kg diet	88.2 (6)	117.7 (6)	0	
.125% thyroid & 2700 mg 2-methyl-2-n-propyl-1,3-propanediol dicarbamate/kg diet	93.9 (6)	126.4 (6)	0	

Values in parentheses indicate No. of animals which survived and on which averages are based.

* In Exp. 1 each group consisted of 10 male rats with avg initial body wt of 57.3 to 56.8 g; in Exp. 2 each group consisted of 10 female rats with avg initial body wt of 46.0 to 46.2 g; in Exp. 3 each group contained 6 female rats with avg initial body wt of 56.0 to 56.4 g.

Tests were subsequently conducted to determine the comparative effects of reserpine, chlorpromazine hydrochloride¶ and 2-methyl-2-n-propyl-1, 3-propanediol dicarbamate** on symptoms of thyrotoxicity in the immature thyroid-fed rat. Findings indicate that whereas reserpine when fed at a level of 20 mg/kg of diet resulted in a 100% mortality within 14 days when fed with 0.125% desiccated thyroid, no deaths occurred in any of the rats fed this dose of thyroid in conjunction with chlorpromazine hydrochloride or 2-methyl-2-n-propyl-1, 3-propanediol dicarbamate, nor was weight increment of rats fed the latter drugs in conjunction with desiccated thyroid significantly less than that of rats fed these drugs without the thyroid (Exp. 3, Table I).

No data are available to indicate the mechanisms whereby doses of reserpine and thyroid, which when administered individually are non-lethal, rapidly result in death when administered concurrently. These results were unanticipated since reserpine has been reported to prevent increase in oxygen consumption in rats(1) and guinea pigs(2) administered thyroid hormone. Further-

¶ Thorazine HCl, Smith, Kline & French Labs., Philadelphia, Pa.

** Miltown, Wallace Labs., New Brunswick, N. J.

more, Kuschke and Gruner's(1) suggestion that reserpine functions as a thyroxine antagonist would lead one to expect that rather than potentiating the toxic effects of thyroid administration reserpine would counteract such effects. In this regard it may be of importance that the dosage of reserpine employed in the present experiment was larger than that employed by others. It is possible, however, that the growth retardation and death obtained when thyroid and reserpine were fed concurrently were due not to a potentiation of thyrotoxicity as a result of reserpine administration but rather a potentiation of reserpine toxicity as a consequence of thyroid feeding.

Summary. Doses of reserpine and desiccated thyroid which were non-lethal to immature rats when administered separately resulted in a 100% mortality within a 2 week period when administered concurrently.

1. Kuschke, H. J., Gruner, H., *Klin. Wchnschr.*, 1954, v32, 563.

2. De Felice, E. A., Smith, T. C., Dearborn, E. H., PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 171.

3. Mayer, S. W., Kelly, F. H., Morton, M. E., *J. Pharmacol. and Exp. Therap.*, 1956, v117, 197.

4. Moncke, C. L., *Medizinische,* (Dec.) 1955, No. 50, 1742.

5. Goodman, J. R., Florsheim, W. H., Tempereau, C. E., PROC. SOC. EXP. BIOL. AND MED., 1955, v90, 196.

6. Ford, R. V., Livesay, W. R., Miller, S. I., Moyer, J. H., *Med. Rec.* (Houston), 1953, v47, 608.

7. Ershoff, B. H., Hershberg, D., *Am. J. Physiol.*, 1945, v145, 16.

8. Ershoff, B. H., *Arch. Biochem.*, 1947, v15, 365.

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Effect of Polycarboxylic Acids on Blood Clotting.* (24292)

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The anticoagulant action of citrate has been known for more than 60 years(1). The similarity in molecular structure of isocitrate and aconitate to citrate suggested that these tricarboxylic acids might also interfere with the clotting mechanism. To test this hypothesis, isocitrate, aconitate and the sodium salts of other polycarboxylic acids were incubated with human blood. Of the acids tested, those with 3 free carboxyl groups were effective in prolonging the clotting time.

Methods. Two ml of venous blood from normal volunteers or from patients without known clotting defects was discharged into tubes containing a solution of 0.5 ml of the appropriate test substance and into control tubes charged with 0.5 ml of 0.85% saline. The tubes were stoppered, inverted several times and placed upright in a rack at room temperature. Clotting time was defined as the interval between termination of mixing and formation of a firm clot which remained at the bottom when the tube was completely inverted. The polycarboxylic acids, obtained from commercial sources,† were put into solution, neutralized to pH 7 (pH Hydrion paper) and made up to volume with distilled water or saline just prior to use. The citric acid content of the commercial *cis*-aconitic acid, *trans*-aconitic acid, dl-Na_3 isocitrate, and tricarballylic acid was found to be less than 0.05%

* Supported by a grant from the Massachusetts Heart Assn.

† Citric acid was purchased from Baker Chemical Co.; *cis*-aconitic acid, dl-malic acid, fumaric acid, tricarballylic acid, malonic acid, dl-isocitric acid lactone and Na_3 isocitrate were purchased from the California Foundation for Biochemical Research, Los Angeles. The source of the other compounds tested was the Nutritional Biochemical Corp., Cleveland, Ohio. *Trans*-aconitic acid, recrystallized from the commercial preparation, was found to be 86% pure by chromatography(2).