of typhoid carriers; however, both methods were in good agreement in the detection of Vi antibody in these sera. The advantages inherent in the Vi hemagglutination procedure are discussed.

1. Felix, A., Krikorian, K. S., and Reitler, R., *J. Hyg.*, 1935, v35, 421.
2. Felix, A., *Lancet*, 1938, v2, 738.
3. Pijper, A., and Crocker, C. G., *J. Hyg.*, 1937, v37, 332.
4. Almon, L., and Stovall, W. D., *J. Lab. Clin. Med.*, 1940, v25, 844.
5. Klein, M., *J. Inf. Dis.*, 1943, v72, 49.
6. Bhatnagar, S. S., Speechly, C. G. J., and Singh, M., *J. Hyg.*, 1938, v38, 663.
7. Horgan, E. S., and Drysdale, A., *Lancet*, 1940, v1, 1084.
8. Webster, M. E., Landy, M., and Freeman, M. E., *J. Immunol.*, 1952, v69, 135.
9. Landy, M., and Webster, M. E., *J. Immunol.*, 1952, v69, 143.
10. Mackenzie, E. F. W., and Taylor, E. W., *J. Hyg.*, 1945, v44, 31.
11. Landy, M., and Lamb, E., *Fed. Proc.*, 1952, v11, 474.

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Effects of p-Aminosalicylic Acid on Thyroid and Adrenal.* (20189)

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Recent clinical observations have indicated that goiter and, rarely, transient myxedema may occur in patients treated with p-aminosalicylate (PAS) over prolonged periods(1-3). Bavin(4) and Kjerulf-Jensen and Wolffbrandt (5) have shown that rats fed PAS developed cellular hyperplasia of the thyroid gland, and Rosenberg(6), in a study of a large group of peroxidase inhibitors, has reported diminished I^{131} uptake in rat thyroid glands after a single parenteral dose of PAS. Similarly, Hanngren (7) has reported a reduction in thyroid I^{131} uptake in 3 patients given a single intravenous injection of this compound.

Investigations over the past few years have suggested that salicylic acid and similar compounds exert a stimulatory effect on the pituitary-adrenal axis. Cronheim *et al.*(8) and Van Cauwenberge *et al.*(9) have shown that the administration of salicylic acid, aspirin and sodium salicylate to rats results in a marked eosinopenia and reduction of adrenal ascorbic acid and cholesterol concentrations. Whereas Cronheim did not observe these changes in the case of PAS, Van Cauwenberge reported a fall in circulating eosinophiles and ascorbic acid and cholesterol concentrations following a single parenteral dose of this compound. Bavin(4) on the other hand, found no eosinopenic response in mice following PAS administration. Because of these conflicting results and the widespread use of prolonged PAS therapy in tuberculosis, it was felt that further evaluation of the effects of prolonged administration of PAS on rat thyroid and adrenal functions was indicated.

Experimental. Male Sprague-Dawley rats weighing approximately 180 g were used. They were kept at a constant room temperature of 80°F, and were fed standard Purina chow diet, modified as indicated. All rats were weighed at the beginning and at the end of the experiment. The animals were divided into 4 groups of 10 rats each. One group of rats was fed 1% powdered PAS in the diet for 2 weeks and control and comparison groups were given a) stock diet alone, b) stock diet containing 0.2% powdered propylthiouracil and c) stock diet plus daily subcutaneous injections of 1 mg of ACTH gel.† At the end of the 2-week period, a tracer dose of I^{131} (6 μc) was injected intraperitoneally into all animals and at the same time food was removed from the cages. Four hours after in-

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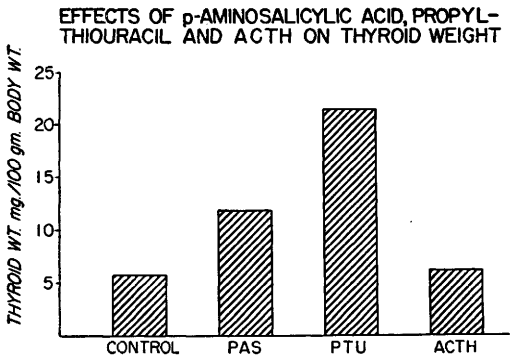

FIG. 1.

jection the animals were anesthetized with ether and blood removed from the aorta. The thyroid glands were weighed and the radioiodine content of the glands was determined by means of a counting system employing a gamma well counter. In addition, the following determinations were made: adrenal weight, adrenal ascorbic acid(10) and cholesterol(11) concentrations; thymic weight, liver glycogen concentration(12) and circulating eosinophiles(13). An experiment similar to the above was carried out on two groups of hypophysectomized male rats.‡ One group received 1% PAS in the diet and the other group served as a control.

Results. All animals studied showed a comparable weight gain and appeared healthy at the end of the 2-week period. PAS given as 1% of the diet, was found to exert a marked goitrogenic effect (Fig. 1), though not as great as propylthiouracil, given as 0.2% of the diet. The thyroid glands of the PAS group averaged 12 ± 0.42 mg/100 g of body weight (mean ± standard error), whereas those of the controls weighed 6 ± 0.27 mg/100 g body weight. This difference is very highly significant ($P < .001$) and constitutes a 2-fold increase in size of the thyroid following PAS therapy. The thyroid glands of the rats given propylthiouracil weighed 3 times as much as those of the controls, averaging 21 ± 0.78 mg/100 g body weight. The weights of the thyroids of the ACTH-treated animals did not vary significantly from those of the controls. The radioiodine concentration in the thyroid glands of PAS group was reduced to 20% of that of the controls, and the total I^{131} content of these hypertrophic glands was 40% of the controls (Fig. 2). No significant difference in thyroid I^{131} concentration was observed between the PAS and propylthiouracil-treated animals, although the larger thyroids of the thiouracil group accumulated more total radioiodine. Again, no significant change was noted in the thyroids of the ACTH group.

Whereas the thyroids of the PAS-fed hypophysectomized animals contained only 17% as much radioiodine as the hypophysectomized controls, no thyroid enlargement was noted, the glands weighing approximately the same in both groups (Fig. 3).

No differences in adrenal weight, adrenal ascorbic acid and cholesterol concentrations, circulating eosinophiles and thymic weight were observed in any of the 4 groups studied (Table I). However, elevation of liver glycogen concentrations in PAS-treated animals was noted. The changes are shown in Fig. 4. Whereas the livers of the controls contained 42 ± 2.25 mg glycogen/g of liver, those of the PAS animals contained 58 ± 3.35 mg/g ($P < .001$). This observation was also made in hypophysectomized animals, where liver

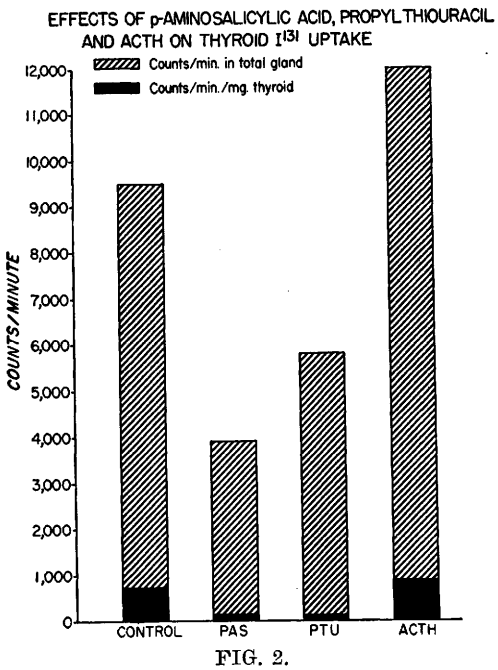

FIG. 2.

‡ Obtained from the Endocrine Laboratories, Madison, Wisc.

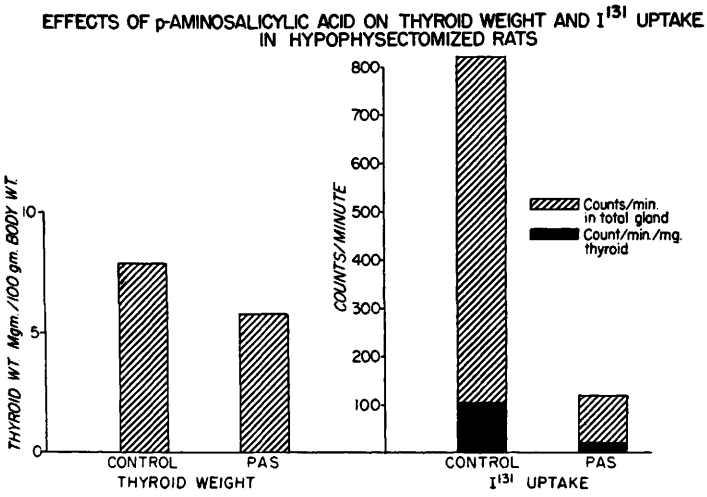

FIG. 3.

glycogen concentrations in the controls were 1.2 ± 0.69 mg/g of liver and those in PAS animals were 19 ± 6.52 mg/g (Fig. 4). This difference is also significant (P 0.02).

Discussion. These studies showed that the administration of PAS results in goiter and inhibition of I^{131} uptake by the thyroid gland in rats, presumably by a mechanism similar to that postulated in the case of propylthiouracil. Inhibition of iodine concentration by the gland results in a decreased formation and secretion of thyroxine with consequent increased secretion of thyrotropic hormone by the anterior pituitary. This in turn, results in thyroid stimulation and enlargement. This explanation is supported by the findings in hypophysectomized animals, where thyromegaly was not observed as a result of PAS ingestion, although diminution in I^{131} was. Further substantiation of this thesis comes from observations by Kjerulf-Jensen and Wolffbrandt that the goitrogenic effect of PAS could be prevented by the administration of thyroid extract. 1% PAS in the diet produced less thyromegaly than did a diet of 0.2% propylthiouracil, indicating that PAS is not as potent a goitrogenic agent as propylthiouracil though the type of pharmacological effect on the thyroid gland is probably the same in both cases. The diminished I^{131} uptake by the thyroid gland following ACTH and cortisone administration reported by Hill *et al.*(14), and Berson(15) was not observed in our experiments. However, it must be kept in mind that the animals in our series received the last injection of ACTH gel 24 hours be-

TABLE I. Effects of *p*-Aminosalicylic Acid, Propylthiouracil and ACTH on Adrenal Properties Function.

Group	Adrenal wt, mg/100 g body wt	Adrenal ascorbic acid, mg/100 mg gland	Adrenal cholesterol, mg/100 mg gland	Thymus wt, mg/100 g body wt	Eosinophil count, per mm³
Control	7.71*	0.483	3.40	206	16.9
PAS	7.52	0.419	3.90	202	17.5
Propylthiouracil	7.43	0.490	3.50	159	14.2
ACTH	9.43	0.655		191	19.2
Hypophysectomized control	4.90	0.364		197	11.7
Hypophysectomized PAS	3.94	0.340		146	23.3

* All values are the mean of individual determinations on 10 animals.

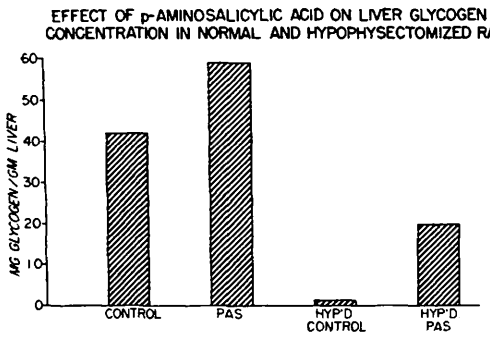

FIG. 4.

fore the radioiodine tracer dose was given.

Our findings substantiate the observation of Cronheim *et al.* that PAS has no effect on the pituitary-adrenal axis, at least when administered over a 2-week period. Although elevation of the liver glycogen concentration was observed in all PAS-treated animals, this is not necessarily an adrenal-mediated response and may be the result of a direct action of PAS in the liver; this is especially interesting in view of the report by Zahn(3) that many patients receiving PAS excrete a reducing substance in their urine. Further work on this phase of the problem is contemplated.

Summary. 1. Administration of PAS to rats over a 2-week period produced goiter associated with diminished I^{131} uptake by the thyroid gland. 2. No effect on the pituitary-adrenal axis was observed. 3. Elevation of liver glycogen concentration was noted in all PAS-treated animals.

1. Clausen, R., and Kjerulf-Jensen, K., *Nordisk Med.*, 1951, v45, 475.

2. Komrower, G. M., *Brit. Med. J.*, 1951, v2, 1193.

3. Zahn, D., personal communication.

4. Bavin, E. M., and James, B., *J. Pharm. and Pharmacol.*, 1952, v4, 856.

5. Kjerulf-Jensen, K., and Wolffbrandt, G., *Acta Pharm. Scand.*, 1951, v7, 376.

6. Rosenberg, I. N., *Science*, 1952, v116, 503.

7. Hanngren, A., *Lancet*, 1952, v263, 117.

8. Cronheim, G., Stanton-King, J., and Hyder, N., PROC. SOC. EXP. BIOL. AND MED., 1952, v80, 51.

9. Van Cauwenberge, H., Bertz, H., Hensghem, C., and Vliers, M., *Annales D'Endocrinologie*, 1951, v12, 788.

10. Roe, J. H., and Kuether, C. A., *J. Biol. Chem.*, 1943, v147, 399.

11. Foldes, F. F., and Wilson, B. C., *Anal. Chem*, 1950, v22, 1210.

12. Good, C. A., Kramer, H., and Somogyi, M., *J. Biol. Chem.*, 1933, v100, 485.

13. Hills, A. G., Forsham, P. H., and Finch, C. A., *Blood*, 1948, v3, 755.

14. Hill, R. S., Reiss, R. S., Forsham, P. H., and Thorn, G. W., *J. Clin. Endocrinol.*, 1950, v10, 1375.

15. Berson, A., and Yalow, R. S., *J. Clin. Endocrinol. and Met.*, 1952, v12, 407.

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A Simple Direct Method for Absolute Basophil Leucocyte Count.* (20190)

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Little is known concerning the physiologic and pathologic significance of the basophil. Previous attempts to study these cells have been hindered by the scarcity of basophils in the bone marrow and circulating blood. Slight differences in absolute basophil counts are not easily detectable by indirect methods and at least 1000 leucocytes should be counted to insure a fair sampling of the basophils present. Such counts are also apt to have a large margin of error due to distribution of leucocytes in the stained blood smear. With a direct counting method, one in which the cells are tallied and differentiated in the same

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