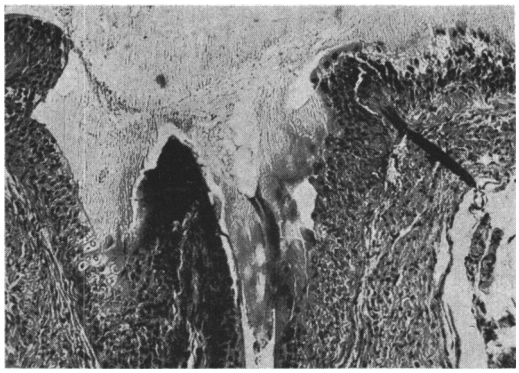


FIG. 2. Photomicrograph of vesicular neck and proximal urethra blocked by proteinaceous plug.

sis was not observed. Interstitial foci of inflammatory infiltrate were seen in many kidneys, and several mice had deposits of amorphous eosinophilic material (presumably amyloid) in glomeruli, spleen and liver. It is not known whether the interstitial nephritis and amyloidosis were related to the urinary obstruction or coincidental. The presumptive cause of death was acute urinary retention.

Several bladder plugs were collected and pooled for chemical analysis. They were negative for calcium, oxylate, phosphate, and uric acid. They were completely digested when left overnight in a 0.2% solution of trypsin and water, indicating that they were composed largely or entirely of protein.

Summary. Proteinaceous bladder plugs are reported as a cause of acute urinary obstruction and death in laboratory mice.

Received September 9, 1965. P.S.E.B.M., 1965, v120.

Examination of Erythrocyte I^{131}-T_4 and I^{131}-T_3 Uptake Test for Thyroid Function in Chickens.* (30596)

MAX O. HUTCHINS (Introduced by Edwin L. Smith)
Department of Physiology, University of Texas Dental Branch, Houston

Recently Hamolsky *et al*(1) have devised an *in vitro* test of thyroid function in humans employing I^{131}-labeled triiodothyronine (T_3). This test of thyroid function is a simple and rapid procedure. It requires only a small sample of blood from the subject, and it yields results of diagnostic accuracy comparable to other tests, such as determination of the basal metabolic rate, serum protein bound iodine (PBI) and thyroid uptake of I^{131}.

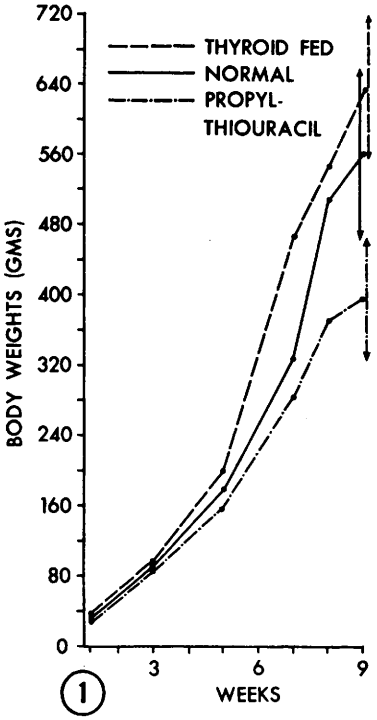
This test has been extensively employed not only in measuring thyroid function in humans(1,2), but also in rats(3) and cattle (4). Since chicken blood differs from these mammals in that it contains no specific thyroxine binding protein(6,7), and has a low serum PBI(5), it was important to evaluate Hamolsky's method as a test for thyroid function in chickens. A second object of this study was to evaluate the observation made by Heninger and Newcomer(8) that there is a difference in binding affinity for T_3 and thyroxine (T_4) by chicken plasma protein, as reflected by this test.

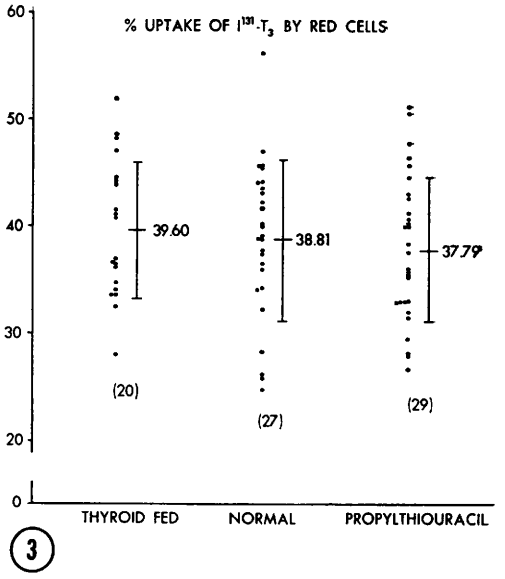
Materials and methods. White Rock cockerels, ranging in age from 1 day to 1 week, were divided into 3 groups and treated as follows: (a) Group I received desiccated thyroid† in their food (0.1 g per 1 kg of food), (b) Group II served as normal controls and were untreated, (c) Group III received propylthiouracil in their drinking water.

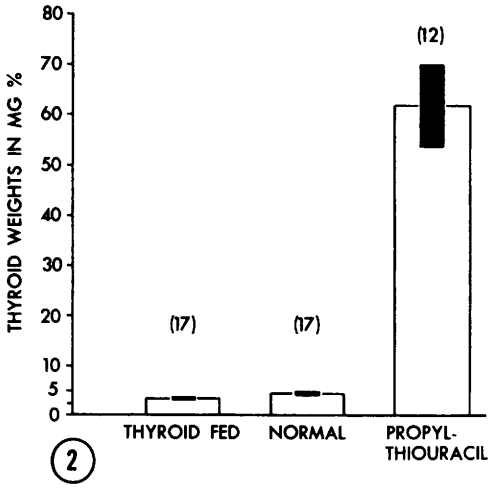
Mean body weights of each group were calculated every 14 days, and at 9 weeks of age the erythrocyte uptake test was conducted. The methods of Hamolsky *et al*(1) were followed throughout, except for certain modifications necessary to adapt them to chickens. Blood was collected by cardiac puncture and 2 ml were placed in each of 2 tubes which contained potassium oxalate. A

* This work has been supported in part by USPHS Grant 1 SO1 FR-05344-01.

† U.S.P. Nutritional Biochemicals Corporation.







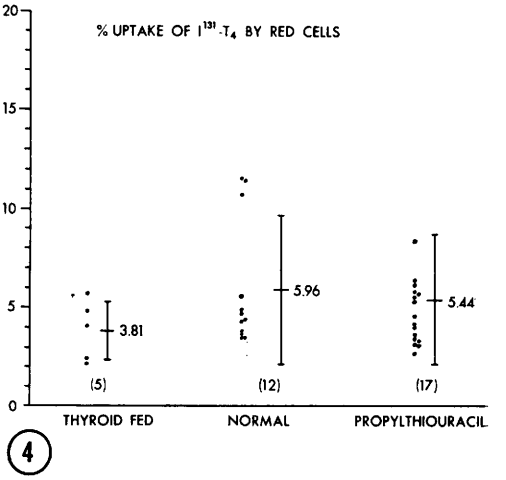


FIG. 1. Mean body weights for each group during a 9-week experimental period. Vertical dotted line indicates standard deviation of mean. Propylthiouracil significantly depressed body weights in these chickens (p .01).

FIG. 2. Mean thyroid weight in mg% for each group at 9 weeks of age. Number of animals used shown at top of each bar. Darkened line indicates standard error of mean. Thyroid fed chickens had a significantly smaller mean thyroid weight than normal controls (3.3 $\pm$ .28 *vs* 4.56 $\pm$ .27 respectively, p .05). Propylthiouracil chickens had significantly larger mean thyroid weight than normal controls (p .01).

FIG. 3. The % uptake of I^{131}-T_3 by red blood cells for each group at 9 weeks of age. Number of animals used shown in parentheses. Mean and standard deviation for each group is given. No significant differences for mean % uptake were noted among the 3 groups.

FIG. 4. The % uptake of I^{131}-T_4 by red blood cells for each group at 9 weeks of age. Number of animals used shown in parentheses. Mean and standard deviation for each group is given. No significant difference for mean % uptake was noted among the 3 groups.

0.1 ml of dilute I^{131}-T_3‡ (20,000 to 40,000 c/m) was added to each tube and the tubes were then stoppered prior to incubation. The incubation temperature (41.5°C) corresponded to the higher body temperature observed in birds(9), instead of to the body temperature of humans. The tubes were gently shaken for 2 hours, then a 1.0 ml aliquot was taken for counting in a well-type scintillation counter. Each aliquot was then suspended in 10 ml of 0.92% saline solution and centrifuged. After 6 of these washings, each tube was recounted. The hematocrit was determined by the microhematocrit method at time of collection of blood and, according to Hamolsky's procedure(1), these values were used to calculate the % erythrocyte uptake of T_3. Duplicate samples from each sample differing by 3% or more were discarded. The thyroids were collected from several birds of each group, and the weights expressed as mg% (mg/100 g body weight).

The above procedure was repeated except that I^{131}-T_4 was added to the blood instead of I^{131}-T_3.

Results. The group of chickens given propylthiouracil had a significantly smaller mean body weight and a significantly larger mean thyroid weight than the thyroid fed or normal control groups (Fig. 1, 2). The chickens that were fed thyroid were not significantly different from the normal control chickens in body weight, but they had a significantly smaller thyroid weight. No other parameters of thyroid function were measured in these chickens with the exception of the *in vitro* test.

The results of the *in vitro* erythrocyte I^{131}-T_3 uptake test are shown in Fig. 3. The 2-hour incubation period employed in this investigation produced no significant differences in the mean I^{131}-T_3 uptake or the mean I^{131}-T_4 uptake of the 3 groups (Fig. 4). The values reported in this study for % uptake of I^{131}-T_3 and I^{131}-T_4 for erythrocytes from normal control chickens were similar to those of Heninger and Newcomer(8), and confirmed their observations that T_3 and T_4 have different binding affinities for the chicken plasma protein, as reflected by Hamolsky's test.

Summary and discussion. From the results of this work the *in vitro* erythrocyte I^{131}-T_3 uptake test was not useful for the measurement on thyroid function in chickens.

Sodee(2) and Szabo(4) pointed out that the outcome of the test was dependent upon avoiding technical variations in the procedure. In the present investigation, temperature was controlled and the same treatment was given to all samples. According to Saxena *et al*(3), the use of propylthiouracil did not appear to alter the erythrocyte's ability to take up I^{131}-T_3; therefore, the use of propylthiouracil to produce hypothyroidism should not have affected the outcome of the test. It is possible that there were no differences in thyroid secretory rate among the 3 groups of chickens, since no parameters of thyroid function (PBI, I^{131} uptake or I^{131} release) were measured. However, since thyroid weights in mg% were significantly different among the 3 groups and the body weights in the propylthiouracil group were significantly low, it is improbable that thyroid function in all 3 groups was the same.

Another contributing factor or factors in the failure of this test may have been the low PBI value reported in avian plasma by Mellen and Hardy(5). Tata and Shellabarger (6,7) also have reported that T_4 and T_3 are not transported in avian sera by the alpha-type globulin found in mammals, but are bound less firmly to the serum albumin fraction. Since in mammals this test actually measures the degree of saturation of several plasma proteins, particularly the globulin fraction with T_3, it is conceivable that in chickens T_3 not bound to plasma proteins would become bound to a greater degree on the erythrocytes. The latter possibility was observed earlier by Heninger and Newcomer (8). It is not clear why the erythrocytes from hypothyroid chickens should not bind less of the added T_3 than erythrocytes from normal controls. Possibly the nucleated character of the avian erythrocytes contributed to the erythrocyte values reported in Fig. 3 and

‡ Various shipments of labeled T_3 and T_4 from Abbott Laboratories contained a mean purity of 86% and 92% respectively, as indicated by single dimensional chromatography in butanol, ethanol, 2N ammonium hydroxide (5:1:2).

4, since Carter *et al*(10) reported that human reticulocytes had a high binding power for T_3.

I^{131}-T_4 was added to the erythrocytes in order to examine the use of this isotope in conducting this *in vitro* erythrocyte uptake test in chickens. Since T_4 was bound firmly to the avian plasma proteins and weakly to the erythrocytes as it is in humans, it was concluded, on the basis of this work, that I^{131}-T_4 also could not be used in estimating thyroid function by the erythrocyte uptake test.

The author wishes to acknowledge the excellent technical assistance of Mrs. D. K. Fleming.

1. Hamolsky, M. W., Stein, M., Freedberg, A. S., J. Clin. Endocrinol., 1957, v17, 33.

2. Sodee, D. B., J. Am. Med. Assn., 1963, v185, 100.

3. Saxena, M., Crawford, J. D., MacGillivray, M. H., Endocrinology, 1964, v74, 415.

4. Szabo, K. T., Mixner, J. P., Proc. Soc. Exp. Biol. and Med., 1964, v115, 521.

5. Mellen, W. J., Hardy, L. B., Endocrinology, 1957, v60, 547.

6. Shellabarger, C. J., Tata, J. R., ibid., 1961, v68, 1059.

7. Tata, J. R., Shellabarger, C. J., Biochem. J., 1959, v72, 608.

8. Heninger, R. W., Newcomer, W. S., Proc. Soc. Exp. Biol. and Med., 1964, v116, 624.

9. Lamoreux, W. F., Hutt, F. B., Poult. Sci., 1939, v18, 70.

10. Carter, A. C., Schwartz, H. L., Kydd, D. M., Kologlu, S., Endocrinology, 1964, v74, 689.

Received July 1, 1965. P.S.E.B.M., 1965, v120.

STATEMENT OF OWNERSHIP AND MANAGEMENT OF THE PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE.
Owner: Society for Experimental Biology and Medicine (A nonprofit organization), 630 W. 168 St., New York City 32
Location of business office: 630 West 168 St., New York City 32
Managing Editor: Walter S. Root, 630 West 168 St., New York City 32
Published at: 104 Liberty Street, Utica, N. Y.
Frequency of issue: Monthly except September.