males ($P<0.001$) and approached having a significant effect in ACTH-treated mice (Table IIE). There can be no question that castration reduced renal weight ($P<0.0001$).

It is difficult to explain the apparent lack of effect of ACTH on the female reproductive tracts in Exp. III, particularly the significantly lower ovarian and uterine weights in adrenalectomized mice, since these results are contrary to those from a large number of earlier experiments(3-5). The failure of ACTH to affect the female reproductive tract cannot be attributed to the lower daily dose of hydrocortisone for two reasons: First, the failure occurred in intact, as well as in adrenalectomized females, and second, hydrocortisone in the doses used does not affect female reproductive function(5).

Contrary to its lack of effect on reproductive organs of females, ACTH decreased reproductive function in males, in this experiment as indicated by the seminal vesicles, presumably by decreasing testicular androgen production. However, this action apparently is limited as testicular weight was unaffected. ACTH may have acted centrally to inhibit gonadotrophin secretion, as suggested by extrapolation from data for females(5).

Summary. In 3 experiments of balanced design the ability of ACTH to produce renal glomerular disease in intact and adrenalectomized male mice was determined. The effects of castration on ACTH-induced glomerulnephritis were studied and the disease in males and females compared. Adrenalectomized mice were given 35 or 50 μg of hydrocortisone-acetate daily. Four units of ACTH daily for 10 days was used. ACTH-induced glomerular lesions exhibit marked increases in intercapillary mesangial material and deposition of intensely PAS+ material in the mesangial matrix and axial capillaries, and peripheralization and ballooning of capillaries. Four I.U. of ACTH daily induced the severest glomerular disease in non-adrenalectomized males, less in adrenalectomized mice and was unaffected by the presence or absence of the testes. ACTH affected the glomeruli of intact and adrenalectomized male and female mice similarly with respect to control values. Two preparations of ACTH, one assaying 60-80 and the other 110 I.U. per mg, produced similar renal disease. It was concluded that ACTH affected both sexes equally; that its effect was greatest in intact and significantly less in adrenalectomized mice.

1. Christian, J. J., J. Endocrinol., 1964, v28, 6.
2. ———, Proc. Royal Soc. Med., 1964, v57, 169.
3. ———, Endocrinology, 1964, v74, 669.
4. ———, ibid., 1964, v75, 653.
5. Christian, J. J., Lloyd, J. A., Davis, D. E., Recent Progr. in Hormone Res., 1965, v21, 501.
6. Christian, J. J., Acta Endocrinol. (Kbh), 1967, v55, 62.
7. Lillie, R. D., Histopathologic technic and practical histochemistry. New York, Blakiston, 1954.

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Comparison of Biological Activity of Injected and Orally Administered L-Thyroxine, L-Triiodothyronine and Thyroprotein in Fowls.* (32390)

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A method for determination of the thyroxine secretion rate in fowls was presented some years ago(1). It involved the graded injection of L-thyroxine (L-T_4) until the release of thyroidal-I^{131} was blocked, indicating that the L-T_4 had stopped the further release of thyrotropic hormone (TSH). This

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method has been used successfully in the comparison of the analogues of L-T_4 in regard to their biological activity in cattle(2) and rats(3).

The same method has been used also in a comparison of the oral and subcutaneous administration of thyroid hormones in cattle (4) and rats(5).

While previous studies comparing the biological activity of L-T_4 and L-triiodothyronine (L-T_3) in fowls by injection have been reported, the oral effectiveness of these hormones and of thyroprotein have not been studied. The object of this report is to present data on the oral effectiveness of these compounds in comparison with their subcutaneous administration.

Materials and methods. Male and female New Hampshire fowls about 4 years old were maintained in individual cages. The assays of thyroidal activity were conducted during a period from October to June (Tables I, II). Unfortunately, air conditioning was not available so the birds were subjected to partial seasonal temperature variation. Sufficient heat was furnished to maintain a minimum temperature of 35°F to prevent water from freezing.

For each comparison, the same group of birds were included, thus eliminating individual variation; however, since the successive assays covered a period of 9 months, seasonal variation could not be eliminated.

The thyroxine secretion rate (TSR) was estimated by the method described(1). L-thyroxine (L-T_4) was injected subcutaneously in increasing increments of 0.25 μg/100 g bw per day. Tapazole (methimazole) was injected daily at a level of 0.5 mg/100 g bw to prevent recycling of I^{131} from the metabolized L-T_4-I^{131}.

L-triiodothyronine (L-T_3) was injected on an equimolar basis in comparison with L-T_4. Thyroprotein is a synthetic thyroactive iodinated casein with the commercial name of Protamone. It is standardized by the manufacturer to contain 1% L-T_4. Its possible L-T_3 content is not known at this time. At the 1% level, 100 μg thyroprotein would contain 1 μg L-T_4.

For the oral administration of L-T_4 and L-T_3, they were diluted in a slightly alkaline saline solution and administered at the rate of 0.1 ml/100 g bw through a stainless steel tube designed for oral administration. Thyroprotein was homogenized in a 50% solution of saline and propylene glycol and given by tube similarly.

Results. The mean TSR of 20 male birds was 0.71 μg L-T_4/100 g bw. When the same 20 birds were injected with equimolar amounts of L-T_3, the mean TSR was 0.32 μg L-T_3/100 g bw. Thus, compared to L-T_4, L-T_3 was 2.21 times as active biologically. When thyroprotein was injected based on a content of 1% L-T_4, the TSR was 0.66 μg L-T_4, or 66 μg thyroprotein/100 g bw. Thus, thyroprotein was 1.08 times as active biologically as L-T_4 (Table I).

In 16 females, the mean TSR was 0.89 μg L-T_4/100 g bw. When the same 16 birds were injected with L-T_3 on an equimolar basis, the mean TSR was 0.59 μg L-T_3/100 g bw. In these females L-T_3 was 1.52 times as active biologically as L-T_4. Thyroprotein required 0.67 μg of L-T_4 equivalent or 67 μg to equal the TSR. In the females thyroprotein was 1.32 times as active biologically as L-T_4.

In the comparison of the biological activity of thyroxine by subcutaneous and oral administration, the males on L-T_4 in two trials showed that L-T_4 was 96 and 89% as active orally as by injection. In a similar comparison with L-T_3 and thyroprotein, the two preparations were 68 and 58%, respectively, as active orally (Table II).

In the females in two trials L-T_4 was shown to be 41 and 68% as active orally as by injection, and L-T_3 was 56% as active. Thyroprotein was observed to be 62% as active orally as by injection.

Since the males showed 58% and the females showed 62% oral activity, it is considered that 60% represents the mean biological activity of thyroprotein when fed as compared to injection in fowls.

Discussion. In earlier studies,(6,7,8) it was reported that L-T_4 was equal to or more active biologically in fowls than L-T_3. However, in a recent study of lines of docile and flighty male birds(9) it was observed that

TABLE I. Comparison of Biological Activity of L-T_4, L-T_3 and Thyroprotein by Subcutaneous Injection.

Sex	No. of birds	Date of assay	Mean TSR in μg/100 g bw	Mean efficiency compared to L-T_4 (%)	Preparation
♂	20	Oct.	.71	100	L-T_4
"	20	Feb.	.32	221	L-T_3
"	20	Dec.	.66	108	Thyroprotein*
♀	16	Oct.	.89	100	L-T_4
"	16	March	.59	152	L-T_3
"	16	Nov.	.67	132	Thyroprotein*

* Based on 1% L-T_4.

TABLE II. Comparison of Biological Activity of L-T_4, L-T_3 and Thyroprotein by Subcutaneous and Oral Administration.

Sex	No. of birds	Preparation	Route	Date of assay	Mean TSR in μg/100 g bw	Route	Date of assay	Mean TSR in μg/100 g bw	Oral effectiveness compared to subcut (%)
♂	18	L-T_4	Subcut	Nov.	.69	Oral	April	.72	96.2
"	17	L-T_4	"	May	.59	"	June	.66	88.8
"	20	L-T_3	"	Feb.	.32	"	March	.48	67.5
"	20	Thyroprotein	"	Dec.	.66	"	Jan.	1.13	58.2
♀	23	L-T_4	"	Oct.	.52	"	Oct.	1.28	40.7
"	16	L-T_4	"	May	.89	"	June	1.30	68.7
"	30	L-T_3	"	March	.58	"	April	1.03	55.9
"	21	Thyroprotein	"	Nov.	.70	"	Dec.	1.13	62.1

L-T_3 was 2.13 and 2.16, respectively, times as active biologically as L-T_4 by subcutaneous injection. In the present study in males L-T_3 was observed to be 2.2 times as active as L-T_4, confirming the earlier observations. However, in females L-T_3 was observed to be only 1.5 times as active as L-T_4. This lower value may be due to seasonal variation in TSR at the time the assays were conducted.

The subcutaneous injection of thyroprotein claimed to contain 1% L-T_4 showed biological activity in males only slightly higher than the equivalent injection of L-T_4; whereas in females, the biological activity was 1.3 times that of L-T_4.

In two comparisons of subcutaneous and oral administration of L-T_4 in males, the oral effectiveness was surprisingly high, 96 and 89% that of injection. In females, the corresponding relation was 41 and 69%. In males, L-T_3 was 68% as effective by oral administration as by injection and in females 56% as effective.

In the comparison of thyroprotein, the males and females showed quite similar relations by the two modes of administration; the males showed 58% and the females 62% effectiveness as compared to subcutaneous administration.

Data are available in two other species, the dairy cow and the rat, comparing the biological effectiveness of L-T_4 and L-T_3 by subcutaneous injection, using the thyroxine secretion rate (TSR) method as a basis of comparison. It was reported that L-T_3 was 2.14 times as effective as L-T_4 by subcutaneous injection(3). In the present study in fowls, the males showed L-T_3 to be 2.2 times as effective as L-T_4 and in females, 1.5 times as effective. No explanation can be offered as to the difference in biological activity reported by others for L-T_4 and L-T_3 in fowls, and that reported here. If further study confirms our observations, the increased biological activity of L-T_3 in the fowl is quite comparable to that observed in the cow and rat.

The oral effectiveness of thyroid preparations in various species differs widely. In ruminant animals such as the dairy cow, it was reported that thyroprotein containing 1% thyroxine was only 10% as effective orally as by subcutaneous injection(10). In a more re-

cent study, these observations were confirmed(4). It was found that thyroprotein was only 9.9% as effective orally as by injection.

In the male rat, thyroprotein containing 1% thyroxine was observed to be 36.2% as effective orally as by injection(5). In the present study, the oral administration of thyroprotein was observed to be 58% as effective in males and 62% as effective in females. Thus, in fowls the oral effectiveness of thyroprotein may be considered to be about 60% of that by injection. Thus, the oral effectiveness of thyroprotein in the fowl is much higher than that in other species studied. This would indicate a higher degree of absorption of L-T_4 from the digestive tract in the fowl.

While thyroprotein has been fed to fowls for many years on an experimental basis with variable results and while the variation in the thyroxine secretion rate (TSR) of fowls has been reported based upon the subcutaneous injection of thyroxine, this is the first report on the oral effectiveness of thyroprotein.

Data indicating that thyroprotein (containing 1% L-T_4) is about 60% as effective orally as by injection makes it possible, for the first time, to estimate the amount of thyroprotein that must be added to the ration to equal the TSR. Since breeds and strains of fowls vary in body weight and feed consumption as well as in thyroxine secretion rate, only the general principles of the estimation can be indicated.

1. Thyroprotein contains 1% L-T_4. Thus a fowl secreting 1.0 μg L-T_4/100 g bw/day would require 100 μg thyroprotein/100 g bw/day by injection or since it is 60% effective orally, it would require 167 μg/100 g bw/day orally. Thus, in a 1, 2 or 3,000 g bird, the daily requirements would be 1.67, 3.34 or 5.01 mg/day to equal their thyroxine secretion rate.

2. Fowls vary in TSR. Some birds secrete only 0.5 μg L-T_4/100 g bw, whereas others may secrete as much as 2, 4 or 6 μg/100 g bw(11). The thyroprotein requirement for a bird secreting 1.0 μg/100 g bw is indicated in 1. Birds secreting more L-T_4 would require multiples of the thyroprotein indicated, based upon body weight.

3. If a hormone is to be effective as a replacement of a deficient secretion rate, it must be administered in excess of the secretion rate. In dairy cattle, the administration of L-T_4 50% in excess of the secretion rate resulted in the maximum increase in milk production(12). In fowls, the feeding of thyroprotein 50% in excess of the mean TSR should be in excess of the secretion rate of most of the birds in the group.

4. In order to incorporate the thyroprotein in the laying ration in the proper amounts, it is necessary to estimate the amount of feed eaten per day. Morrison(13) gives an estimate of the daily feed intake of light and heavy breeds of fowls based upon their egg production. Thus, for the light breeds weighing an average of 4 lbs (1814 g) at 60% production, the average daily feed consumption is 0.28 lbs (127 g).

5. With these data it is possible to estimate the amount of thyroprotein to add to the feed for birds of varying TSR. For fowls with a mean TSR of 1 μg/100 g bw, the daily requirement is 167 μg/100 g bw. To increase the level 50% would be to increase it to 250.5 μg or 4.5 mg for a 1800 g light breed hen. This amount of thyroprotein would be mixed in 113.4 g of feed or 1.8 g/100 lbs or 36 g/ton of feed. For the heavy breeds weighing 2500 g, the daily level would be 6.3 mg in 127 g of feed or 2.25 g/100 lb or 45 g/ton of feed. For fowls having mean TSR of 2, 3 or 4 μg/100 g bw, the thyroprotein requirement would be multiples of these values.

Summary. Male and female fowls of the New Hampshire strain, about 4 years old, were subjected to diurnal and seasonal variation in temperature. The thyroxine secretion rate (TSR) was first determined using L-thyroxine (L-T_4), followed by L-triiodothyronine (L-T_3) and thyroprotein (containing 1% L-T_4) injected subcutaneously.

In the male birds, if the mean TSR of L-T_4 is considered 100%, L-T_3 was observed to be 2.2 times as active biologically, whereas thyroprotein was only 1.1 times as active. In the females, L-T_3 was 1.5 times as active as

$L\text{-}T_4$, and thyroprotein was 1.3 times as active (based on 1% $L\text{-}T_4$ content) as $L\text{-}T_4$.

In a comparison of subcutaneous and oral administration of these hormones in the male and using the subcutaneous administration as 100%, in two trials, $L\text{-}T_4$ orally was 96.2 and 88.8% as active. $L\text{-}T_3$ was 67.5% as active and thyroprotein, 58.2%. In the females, in two trials, $L\text{-}T_4$ was 40.7 and 68.7% as effective orally; $L\text{-}T_3$ was 55.9% as effective orally; and thyroprotein was 62.1% as effective. Since the oral effectiveness of thyroprotein in male and females is about 60%, it is suggested that this value be used in estimating the amount of thyroprotein to add to a poultry ration to equal the thyroxine secretion rate.

1. Pipes, G. W., Premachandra, B. N., Turner, C. W., Poultry Sci., 1958, v37, 36.

2. Premachandra, B. N., Pipes, G. W., Turner, C. W., Am. J. Physiol., 1961, v201, 77.

3. Bauman, T. R., Pipes, G. W., Turner, C. W., Endocrinology, 1965, v76, 537.

4. Bauman, T. R., Turner, C. W., J. Dairy Sci., 1965, v48, 1353.

5. ———, Proc. Soc. Exp. Biol. & Med., 1966, v123, 9.

6. Shellabarger, C. J., Poultry Sci., 1955, v34, 1437.

7. Newcomer, W. S., Am. J. Physiol., 1957, v190, 413.

8. Mellen, W. J., Wentworth, B. C., J. Poultry Sci., 1959, v38, 228.

9. Srivastava, L. S., Turner, C. W., Proc. Soc. Exp. Biol. & Med., 1967, v124, 325.

10. Premachandra, B. N., Pipes, G. W., Turner, C. W., Mo. Agr. Exp. Sta. Res. Bull., 727, 1960.

11. Stahl, P., Turner, C. W., Poultry Sci., 1961, v40, 239.

12. Hindery, G. A., Turner, C. W., J. Dairy Sci., 1965, v48, 596.

13. Morrison, F. B., Feeds and Feeding, Morrison Publ. Co., Ithaca, N. Y., 1956.

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Factors Influencing Growth Inhibition of *Mycoplasma pneumoniae* By Immune Sera.* (32391)

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Recognition of the importance of *Mycoplasma pneumoniae* as a cause of human respiratory disease has stimulated the development of a variety of methods for detecting a serologic response to this agent(1). Each technique reveals a characteristic response pattern depending on its ability to detect the different immunoglobulins (Ig)(2). Of the biologic properties demonstrable in sera following infection or immunization with *M. pneumoniae,* only growth inhibition appears to derive significantly from all 3 classes: IgM, IgA and IgG. Growth inhibition (GI) has been quantitated directly by determining a decrease in numbers of viable organisms in the presence of homologous antiserum and indirectly by metabolic-inhibition techniques (3,4,5,6). This phenomenon has received particular attention because of its specificity and its tendency to persist in serum for prolonged periods. However, GI methods generally have been technically difficult to perform and standardize, and have been utilized infrequently. To provide insight into the mechanisms of immune inhibition, the dynamics of the GI reaction and variables affecting its expression were studied.

Materials and methods. All procedures utilized a broth medium(7) composed of 70% Difco PPLO broth, 20% unheated horse serum, 10% yeast extract, 1% dextrose, 0.004% phenol red and 1000 units of penicillin G/ml. The broth base was pretested for ability to

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