7 LPS was different with the 3 species of C, the difference between the most and least active within each species of C was of the order of 10–20fold. The serum obtained from 4 of the laboratory staff showed there was no substantial difference between different samples of sera in their responses to the different LPS. Recent work[11,15] has amply demonstrated that LPS from different bacteria may vary widely in AC activity, while the AC activity of a given LPS may be altered by various chemical treatments[16]. The results here however show a striking difference between human and guinea-pig C in their sensitivity to the AC activity of LPS, the human being capable of detecting approximately 200 times lower concentrations of LPS than the guinea-pig. The underlying basis of this difference remains to be explained. LPS may exert its AC effect by both the classical and alternative pathways[17,18] and we have not yet investigated which way predominates under the conditions used. As compared with other methods for assaying LPS, the AC test has the advantages of cheapness, convenience and reproducibility. However, it would appear to be of the order of 1000fold less sensitive than the rabbit pyrogenicity test or the *Limulus* amoebocyte lysate assay[20]. Nevertheless, in certain defined circumstances, AC activity can provide a cheap and convenient assay for LPS and, in work to be reported elsewhere, we have used it to study a) the biodegradation of LPS by the slime mould *Physarum polycephalum* and b) the distribution and fate of LPS in marine sediments.

1 S.E. Greisman and R.B. Homick, Proc. Soc. exp. Biol. Med. *131*, 1154 (1969).
2 W.M. Wolff, J.H. Mulholland and B. Ward, J. Lab. clin. Med. *65*, 268 (1965).
3 R.T. Smith and L. Thomas, J. expl. Med. *104*, 217 (1956).
4 K.C. Milner and R.A. Finkelstein, J. infect. Dis. *116*, 529 (1976).
5 J.F. Cooper, J. Levin and U.N. Wagner, J. Lab. clin. Med. *76*, 138 (1971).
6 A.W. Bernheimer (ed), Mechanisms in Bacterial Toxinology, p. 217. Wiley & Sons, New York 1976.
7 L. Pillemer, M.D. Schoenberg, L. Blum and L. Wurz, Science *122*, 545 (1955).
8 H. Gewurz, S.E. Mergenhagen, A. Nowotny and J.K. Phillips, J. Bact. *95*, 397 (1968).
9 S.E. Mergenhagen, R. Snyderman, H. Gewurz and H.S. Shin, Curr. Top. Micro. *50*, 37 (1969).
10 C. Galanos, E.Th. Rietschel, O. Luderitz and O. Westphal, Eur. J. Biochem. *19*, 143 (1971).
11 O. Westphal, O. Luderitz, E. Eichenberger and W. Keiderling, Z. Naturforschg. *7b*, 536 (1952).
12 G. Keloti and W.H. Lederer, in: Handbook of Micromethods for the Biological Sciences, p. 3. Van Nostrand Reinhold Co., New York 1974.
13 E.A. Kabat and M.M. Mayer, in: Experimental Immunochemistry, 2nd edn., p. 97. Charles C. Thomas, Illinois 1961.
14 H.G. Macmorine, A.C. Wardlaw and J.C.W. Weber, J. Immun. *94*, 611 (1965).
15 M. Loos, D. Bitter-Suermann and M. Dierich, J. Immun. *112*, 935 (1974).
16 C. Galanos and O. Luderitz, Eur. J. Biochem. *65*, 403 (1976).
17 D.P. Fine, J. Immun. *112*, 763 (1974).
18 D. Bitter-Suermann, V. Hadding, H.U. Schorlemmer, M. Limbert, M. Dierich and P. Dukor, J. Immun. *115*, 425 (1975).
19 K. Rother, U. Hadding and G. Till, Komplement Biochemie und Pathologie. Steinkopf Press, Darmstadt 1974.
20 J. van Noordwijk and Y. de Jong, J. Biol. Standard *4*, 131 (1976).

Estimation of plasma thyroxine concentration in ducks in relation to different environmental and experimental conditions[1]

H.S. Astier, J.Y. Daniel and M. Jallageas

Department of Physiology, University of Montpellier II, Place Eugène-Bataillon, F-34060 Montpellier (France), 12 May 1977

Summary. Plasma thyroxine concentration was measured in ducks by the thyroxine-binding globulin technique. The assay allowed us to detect annual variations in thyroid activity as well as significant changes after starvation or cold exposure. No detectable thyroxine was formed in surgically thyroidectomized ducks.

In birds, as in other Vertebrates, protein bound iodine has been routinely used for the measurement of thyroid activity. Using these methods, Mellen and Hardy[2], and Mellen[3] have claimed that protein bound iodine ($PB^{127}I$, as well as $PB^{131}I$) was not significantly altered in chickens after cold stress, thiouracile-treatment, or thyroidectomy. Similarly, we were unable to detect any modification in $PB^{127}I$ of ducks reared for 1 or 30 days in a cold environment (+4 °C) or exposed to reduced (50%) or solid starvation[4].

More recently, using Sephadex column fractionations, we could show that in ducks, hens, pigeons and quail, the $PB^{125}I$ was not restricted to hormonal fraction (Astier[5–7]). A non-hormonal radio-iodinated and organic fraction (TCA precipitable but not extractible with N butanol) appeared in the plasma, preceding largely hormonal radio iodinated compounds and representing between 98 and 100% of the PB*I. The non-hormonal fraction decreased at 24 h after tracer injection, but between 1 and 14 days it was still equal to 30–50% of the PB*I. In addition, stable iodine determination confirmed the presence of 50% of non-hormonal iodinated proteins in the duck $PB^{127}I$.

The present studies were designed to estimate whether plasma thyroxine measurements, with the thyroxine-binding globulin (TBG) technique, could lead to a more accurate evaluation of thyroid function in ducks, and therefore allow the detection of variations in plasma thyroid hormone level in relation to various environmental conditions, even when PBI measurements failed to show any modification. The TBG method used here has already been used as a reliable assay for thyroid hormone concentration in chickens[8,9].

Material and method: The competitive protein-binding procedure used here was adapted from the original assay by Murphy et al.[10] and modified by Vigouroux[11].

Standard thyroxine solution: The standard curve was prepared by pipetting 20–60 µl of the working solution (10 ng/ml of L thyroxine dissolved in 95% ethanol) into polystyrene tubes and evaporating to dryness, under nitrogen at 37 °C.

Labelled thyroxine: L-^{125}I thyroxine (CEA Saclay, France; Sp. act. 40–50 mCi/µg), was dialyzed with rat plasma

against 1 L phosphate buffer during 24 h at 4 °C, and dissolved in 5 ml propylenglycol, 5 ml 9% saline.

Resin: Rexyn 202, (Fisher Scientific Co) soaked in a barbital buffer (pH 8.6, ionic strength 0.75) was completely dried under vacuum before use.

Labelled TBG solution: 1 ml human plasma was added to 50–65 ng dialyzed ^{125}I L thyroxine, and completed to 200 ml with Murphy and Jachan solution. The large TBG dilution used here allowed thyroxine estimations with a precision of 0.1 ng/100 µl plasma.

Extraction procedure and assay: Extraction was obtained with 300 µl fresh plasma and 4 vol. of ethanol (into polystyrene test tubes). After vortex shaking and centrifugation, 0.5 ml of supernatant was evaporated in duplicate under nitrogen in a water-bath at 37 °C, along with thyroxine standards test tubes. After complete evaporation, 0.5 ml of labelled TBG solution were added and tubes incubated at 37 °C (15 min), with 3 strong agitations. All tubes were then transferred to 0 °C for at least 30 min. 0.2 ml vol. of dried Rexyn was added to each tube with an automatic distributor. The tubes were shaken for 2 min, and 2.5 ml of barbital buffer were added simultaneously to each tube. The supernatant was sucked by aspiration and radioactivity bound to Rexyn (free T4) was counted, along with 0.5 ml of labelled TBG solution in an Auto-Gamma Spectrophotometer (Packard). Standard curves were expressed with the ratio of total radio-activity/bound radioactivity as a function of stable thyroxine supplementation.

Animals. Adult Peking ducks (2500–2800 g) were fed a commercial diet containing 2.5 µg iodine/g, and kept outdoors unless specified. Blood samples were obtained by puncture from a leg vein, either in December (group I_a as winter controls) or in July (group I_b as summer controls). From the control group I_a, 5 animals were starved for 17 days (group II), and 5 others were kept at 25 °C for 3 weeks (group III), then exposed to +4 °C for either 1 day (group IV), or 30 days (group V). Blood sampling occurred at the end of each experimental treatment.

Surgical thyroidectomy was performed after nembutal anesthesia and blood samples were obtained, 8 days or 1 month respectively after surgery (groups VI a and VI b). For comparison, plasma thyroxine was also measured in male adult Wistar rats kept at 25 °C for 3 weeks and fed a stock diet (0.60 µg iodine/g).

Results and discussion. Results are listed in the table.

Thyroxine plasma levels in untreated ducks. First of all, a 10fold difference appeared between plasma thyroxine levels in ducks (5.1 ng/ml) and in rats (52.0 ng/ml). PBI has already been found to be lower in birds than in mammals (Astier[6]) and has been explained by the absence of a thyroxine-binding globulin in the plasma of birds[12]. The plasma T4 concentrations obtained here are in agreement with earlier data, where T4 levels in ducks were inferred from the difference between chemically measured iodine in PB I and non-hormonal iodinated protein[6]. These results are close to the T4 level values reported by Refetoff et al.[13], May et al.[8] and Newcomer[9] in adult chickens. In contradistinction, Sadovsky and Bensadoum[14] reported in the rooster T4 values that were as high as those found in the rat, using a quite different method of measurement.

On the other hand, our data demonstrated annual variations in thyroid function, since the plasma thyroxine level appeared to be 3fold higher in July than in December (the difference is highly significant). These results confirm our former findings on seasonal variations in thyroid activity of ducks[15], and the peak shown in July can be interpreted as a consequence of testis-thyroid interactions[15,16].

Effects of starvation. 17 days of starvation induced a 50% decrease in the plasma thyroxine level ($p<0.01$ between group II and group I_a), whereas PB^{127}I determination had failed to show any significant difference[4]. These findings are in agreement with previous data that showed a lowered iodine turnover of intrathyroidal iodinated amino acids after starvation[6]. There are only very few bibliographic data on the effects of malnutrition on the bird thyroid. Premachandra and Turner[17] have emphasized that underfeeding due to reserpine-treatment induced a decrease in thyroxine secretion rate in pigeons.

Effects of cold. As stated above, the cold exposed ducks (groups IV and V) were preadapted to a warm environment of +25 °C. After 1 day exposure to +4 °C, the T4 plasma level rose by 20% ($p<0.05$ between group III and IV). The increase in plasma T4 was 50% after 8 days of cold exposure ($p<0.01$ between group V and III). These results point to a marked stimulation of thyroid function by acute cold exposure, that is comitent with the acceleration of the iodine turnover at the thyroid level, the shortening of the half-life of radio-T4 and the increase of the thyroid secretion rate in ducks reared under similar conditions[6].

Very little information is available on the thyroid response to cold in birds. Hoffman and Shaffner[18], Mueller and Amezcua[19], and Stahl et al.[20] have claimed an increase in thyroidal activity to occur in hens and cockrels after 1 or several weeks and even months of cold exposure, while Hendrich and Turner[21] have shown an increase in radioiodine uptake after 1 or 3 days of cold exposure in hens. Recently, Kuhn and Nouwen[22] found an increase in both T3 and T4 after 2 and 3 days of a mild cold stress in the domestic fowl. In spite of the diversity of methods used in thyroidal investigation, exposure to cold can therefore be regarded as a major environmental stimulus to the thyroid function in birds.

Effects of surgical thyroidectomy. The table shows that 8 days or 1 month after surgical removal of both thyroid lobes, no detectable amount of thyroxine could be measured in plasma from thyroidectomized ducks. These results may appear to be in marked contradistinction with Mellen's[3] statements, who found that the conversion ratio

Effects of various environmental and experimental conditions on plasma thyroxine concentration in drakes. Comparison with the rat

Groups and conditions	Number of animals	Plasma T4 (ng/ml)
Rat (Wistar)	6	52.0±0.3
Peking duck		
Group I_a (controls in December)	5	5.1±0.3
Group I_b (controls in July)	5	14.6±0.7*
Group II (17 days starvation)	5	2.4±0.4*
Group III (3 weeks at 25 °C)	5	7.9±0.4
Group IV (1 day at 4 °C)	5	9.5±0.2***
Group V (8 days at 4 °C)	3	13.5±0.7**
Group VI (before thyroidectomy)	6	6.8±0.7
Group VI_a (8 days after Thyr)	6	Undetectable
Group VI_b (1 month after Thyr)	6	Undetectable

*$p<0.01$ vs group I_a; **$p<0.01$ vs group III; ***$0.01<p<0.05$ vs group III.

($PB^{131}I\%$) in thyroidectomized chickens was as high as in intact controls, 72 and 96 h after a radioiodine injection.
However, in recent experiments (unpublished data) using gel Sephadex column fractionation, we could show that in thyroidectomized ducks: protein-bound radioactivity was still present in the plasma but this PBI included 100% of non-hormonal iodine. It therefore seems unquestionable that total thyroidectomy removes the only source of thyroid hormone in birds as in other species.
Conclusion. The competitive protein-binding procedure appeared to provide a reliable method for the study of avian thyroid function. The results presented here on drakes showed (a) low levels in plasma T4 compared with the rat, (b) striking seasonal changes in animals reared outdoors, (c) marked variations in animals exposed to environmental conditions that can be considered as rather common for wild life animals, e.g., temporary deprivation of food (i.e. during migration or egg incubation), and cold. Whereas PBI was unable to detect any modification in thyroid function, the T4 binding method showed significant alterations of thyroid activity in such circumstances. And finally, the method can be useful in testing the effectiveness of thyroidectomy, while PBI determinations are definitely inadequate in this respect.

1 Supported by CNRS and the Department of Biology of the CEA.

2 W.J. Mellen and L.B. Hardy, Endocrinology *60*, 545 (1957).
3 W.J. Mellen, Gen. comp. Endocr. *10*, 315 (1968).
4 H. Astier, N. Mas-Jougla and I. Assenmacher, C.r. hebd. Acad. Sci., Paris *275*, 2531 (1972).
5 H. Astier, C.r. hebd. Acad. Sci., Paris *276*, 793 (1973 a).
6 H. Astier, Thèse. Université de Montpellier No. CNRS AO 8721 (1973 b).
7 H. Astier, Comp. Biochem. Physiol. *52 A*, 9 (1975).
8 J.D. May, L.F. Kubena, J.W. Deaton and F.N. Reece, Poultry Sci. *52*, 668 (1973).
9 W.S. Newcomer, Gen. comp. Endocr. *24*, 65 (1974).
10 B.P. Murphy, C.J. Pattee and A. Gold, J. clin. Endocr. *26*, 247 (1966).
11 E. Vigouroux, C.r. hebd. Acad. Sci., Paris *275*, 579 (1972).
12 J.R. Tata and C.J. Shellabarger, Biochem. J. *72*, 608 (1959).
13 S. Refetoff, N.I. Robin and V.S. Fang, Endocrinology *86*, 793 (1970).
14 R. Sadovsky and A. Bensadoun, Gen. comp. Endocr. *17*, 268 (1971).
15 H. Astier, F. Alberg and I. Assenmacher, J. Physiol., Paris *62*, 219 (1970).
16 M. Jallageas, H. Astier and I. Assenmacher, Gen. comp. Endocr. *34*, 68 (1978).
17 B.N. Premachandra and C.W. Turner, Proc. Soc. exp. Biol. Med. *104*, 306 (1960).
18 E. Hoffman and C.S. Shaffner, Poultry Sci. *29* 365 (1950).
19 W.J. Mueller and A.A. Amezcua, Poultry Sci. *38*, 620 (1959).
20 P. Stahl, G.W. Pipes and C.W. Turner, Poultry Sci. *40*, 646 (1961).
21 C.E. Hendrich and C.W. Turner, Poultry Sci. *42*, 1190 (1963).
22 E.R. Kühn and E.J. Nouwen, Gen. comp. Endocr. *34*, 336 (1978).

Possible influence of prolactin on intestinal hypertrophy in pregnant and lactating rats[1]

J.R. Mainoya[2,3]

Department of Zoology and Cancer Research Laboratory, University of California, Berkeley (California 94720, USA), 23 January 1978

Summary. The increase in intestinal weights during lactation, and to a lesser extent during pregnancy, is inhibited by bromocriptin. This suggests that increased prolactin secretion might be responsible for gut hypertrophy during lactation.

It is now evident that gestational and lactational states may modify intestinal absorption of electrolytes and nonelectrolytes[4-6]. Recently, it was reported that rat intestinal dipeptidase activity increased slightly during pregnancy and much more during lactation[7]. Available data indicate that the small intestine shows important increases in weight in lactation[4,5,8-10] which are not always observed during pregnancy[4,5]. Some observations suggest that during lactation intestinal weight and length progressively increase to a maximum a few days before weaning and decrease to normal values about 30 days post partum[5,9].
Although there is a definite relationship between gut weight changes and the period of lactation[5,9], little is known about the mechanisms which control gut hypertrophy during lactation. Increased food intake has been attributed to the observed gut hypertrophy and hyperplasia[9]. Recently, it has been suggested that the effects of pregnancy and lactation on gut functions, such as gut hypertrophy observed during lactation, could be mediated by prolactin[11]. The purpose of the present note is to report on the results of an investigation to find out whether the gut hypertrophy seen during lactation is attributable to increased prolactin secretion.
Materials and methods. In the present study, nonfasted 3-month-old Sprague Dawley rats (Simonsen Labs. Gilroy, California) were used. They were maintained on a 'white diet' (Simonsen Lab.), food and water given ad libitum.
The groups of rats used included: Dioestrous rats, rats at day 12-13 of pregnancy, mid-pregnant rats pretreated once daily for 5 days with 60 µg of 2-bromo-α-ergocryptine (= bromocriptin or CB-154, Sandoz), rats between day 5 and 6 of lactation nursing 8 pups, and rats nursing 8 pups pretreated once daily from day 1 of p.p. for 5 days with 60 µg of CB-154. Bromocriptin (60 µg) was suspended in physiological saline and injected s.c. to mid-pregnant and lactating rats.
Following nembutal anaesthesia, 2 12-cm long segments from the mid-jejunum (segments III and IV) were removed, rinsed out with physiological saline, quickly blotted on filter paper and weighed on a Microanalytical Mettler-H-balance. Each litter was weighed and average weight of pup determined per lactating rat. The weights are given in g and expressed as means ± SEM. All statistical comparisons were made with a t-test.
Results. As shown in table 1, jejunal weight increases during mid-pregnancy only reached significant levels in segment IV ($p < 0.05$). Pregnant rats pretreated for 5 days with CB-154 had jejunal weights which did not differ from dioestrous controls. However, 5 days lactation caused significant increases in both segments ($p < 0.001$) as compared with dioestrous rats. But lactating rats treated with CB-154 from day 1 p.p. for 5 days showed significant decreases in jejunal weights compared with untreated lactating rats ($p < 0.01$).