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INCREASED ^{131}I UPTAKE BY THE THYROID GLANDS OF OBESE STRAIN (OS) CHICKENS DERIVED FROM NON-PROTAMONE-SUPPLEMENTED HENS

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SUMMARY

The Obese strain (OS) of chickens spontaneously develops autoimmune thyroiditis several weeks after hatching, characterized by severe lymphoid infiltration and circulating thyroglobulin antibody. Sundick & Wick (1974) found that the thyroid glands of OS embryos and newly hatched chicks actually incorporated more ^{131}I than normal controls when the parents of both groups were fed a Protamone-supplemented diet. Since this material—an iodinated casein containing thyroactive substances—drastically reduced thyroidal uptake of ^{131}I, several experiments were designed to compare OS and normal chicks free from these effects. In the first experiment the dietary supplementation of OS and normal hens were changed from Protamone to pure thyroxine and triiodothyronine. Their eggs were collected daily and incubated. The hatched chicks were tested for 20-hr ^{131}I uptake and it was determined that 11–18 days after the food switch, the inhibition of uptake by Protamone was reversed, and the newly hatched OS chicks still had a significantly increased 20-hr ^{131}I uptake when compared to the normal controls. Comparison of the offspring of a special flock of OS hens that lays without hormonal supplementation, with the parental Cornell C strain from which the OS is derived, similarly revealed a higher 20-hr ^{131}I uptake by the OS ($P<0{\cdot}005$). The increased thyroidal uptake of OS was apparent as early as 4 hr after ^{131}I administration and seemed to be independent of slight variations in the amount of ^{127}I available to the chicks, and also seemed to be independent of maternally derived thyroglobulin antibody vertically transferred into OS chicks.

These results suggest that an abnormality of the OS thyroid gland might be a prerequisite for the spontaneously occurring autoimmune thyroiditis in this strain.

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INTRODUCTION

OS chickens are afflicted with a severe spontaneously occurring autoimmune thyroiditis (SAT) (Cole, Kite & Witebsky, 1968; Witebsky *et al.*, 1969; Wick, Sundick & Albini, 1974). Many features of this disease are quite similar to those of human Hashimoto's thyroiditis. These include circulating thyroglobulin autoantibodies, lymphoid infiltration of the thyroid glands with characteristic formation of many germinal centers, and interestingly, a prevalence of the disease among females. Due to these similarities and the many possibilities for immunological experimentation provided by avian species, this strain appears to be a useful model for thyroiditis and perhaps other autoimmune diseases as well.

The question of whether the development of SAT is caused by a primary abnormality of the immune system or of the target organ has not yet been clearly answered. Studies of Hashimoto's thyroiditis provided evidence for abnormalities of both the target organ and the immune system. A primary thyroid defect is inferred by the presence of abnormal thyroid function (an increase of non-butanol extractable iodine) in the sera of some patients and often also their relatives (Vanhaelst *et al.*, 1972). Evidence for a disturbed immune system comes from studies demonstrating an increased incidence of autoimmune reactivity also to a variety of non-thyroid antigens (Delespesse *et al.*, 1972). One criticism of the thyroid function studies in Hashimoto's patients is the inability to distinguish cause from effect, i.e. abnormal thyroid function may have been present before lymphoid infiltration, or it may have developed as a sequel to the latter. In OS chickens this question seemed relatively easy to answer, since the thyroid are not infiltrated by lymphoid cells during the neonatal period and then more than 90% of the chickens quickly develop autoimmune thyroiditis within several weeks.

In a previous study data were presented in which thyroid glands of OS embryos and newly hatched chicks were shown to incorporate significantly more ^{131}I than normal White Leghorn (NWL) controls (Sundick & Wick, 1974). This suggested the presence of a thyroid abnormality in the OS prior to lymphoid infiltration. One criticism of these studies was the diet used for the breeders; to provide adequate thyroxine (T_4) for these hypothyroid OS chickens, which lay poorly without it, Protamone® was added to the diet. Protamone is produced commercially by the iodination of casein, resulting in the formation of a compound containing T_4, iodinated carbon constituents not biologically active, and free iodide. Some of the substances are transferred into the egg, causing a considerably reduced uptake of ^{131}I by the thyroid glands of the offspring. Although this factor was controlled by feeding the identical diet to the normal breeding chickens, further studies seemed necessary to exclude the substances in Protamone as factors causing the OS to incorporate significantly more ^{131}I than NWL controls. Accordingly, in one study Protamone was replaced by pure T_4 and triiodothyronine, and in another one OS chickens selected for their ability to lay without hormonal supplementation were compared to appropriate controls. The possible effects of thyroglobulin antibodies, transferred vertically from the hen to the egg (Kite *et al.*, 1969), on thyroidal uptake of ^{131}I was also investigated.

MATERIALS AND METHODS

Protamone in diet replaced by pure T_4 and T_3

In this experiment (performed in July, 1973) six OS hens and one OS rooster, about 1·5

years of age, were placed in a large pen with metal screening underneath. They were fed normal laying feed (Assmann-Muehle, Vienna, Austria) supplemented with 0·01% Protamone (AgriTech, Incorporated, Kansas City, Missouri). Seven hens and one rooster derived from NWL eggs purchased locally were fed and raised under identical conditions. Several months after steadily receiving Protamone-supplemented food, the diet was abruptly changed; instead of Protamone, purified L-thyroxine (T_4) and L-3, 5, 3′-triiodo-thyronine (T_3) were added to the feed (Combithyrex® forte tablets, Sanabo Ges.m.b.H., Vienna). The tablets were ground up and thoroughly mixed into the feed, yielding a final concentration of 20 μg of T_4 and 5 μg of T_3/100 g of feed. The daily intake of T_4 and T_3 of our chickens was equivalent to the estimated thyroid excretion rate of normal chickens, assuming that they consumed 100 g of feed daily, that T_4 and T_3 are of equal potency in chickens (Raheja & Snedecor, 1971) and that normal chickens have a daily excretion rate of 1–2 μg of T_4/100 g of body weight (Falconer, 1971).

Eggs layed by these hens were collected daily, dated and stored at 16°C for no longer than 2 weeks before incubation. OS and NWL eggs were incubated simultaneously at 37·5°C and automatically rotated every 6 hr. Within 24 hr of hatching each chick was injected intraperitoneally (i.p.) with 1 μCi of carrier-free Na ^{131}I (The Radiochemical Centre, Amersham, Buckinghamshire, England) diluted in 0·5 ml phosphate-buffered saline (PBS), pH 7·2. Before injection this quantity had a radioactive emission level of 660,000 cpm as measured in a Phillips PW 4002 well-type gamma scintillation counter. The chicks were sacrificed 20 hr later by ether inhalation and the two thyroid lobes were removed and analysed for uptake of ^{131}I in the gamma counter. Background counts were negligible.

The effect of T_4 and T_3 replacement of Protamone feeding of hens on the total iodine content of the egg was determined by Professor Dr H. Zacherl (Institute for Medicinal Chemistry, Veterinary School, Vienna) using a Technicon autoanalyser. Eggs to be analysed had been laid 19–21 days after the change of food. For control purposes eggs were collected from other OS hens maintained on a Protamone-supplemented diet as usual.

Comparison of OS and Cornell C strain chicks

While most OS hens require thyroxine supplementation for adequate egg production, others maintained by Dr R. K. Cole at Cornell University still lay reasonable numbers of eggs without exogenous thyroxine. Fertile eggs collected from these hens were transported to Vienna by air freight for use in these studies. Control eggs collected from C strain chickens were simultaneously collected and shipped. Both groups of eggs were therefore obtained from hens fed a normal laying diet without hormonal supplementation. C strain chickens appeared to be an ideal control since the OS was actually developed by selective breeding of those few (less than 1%) C strain chickens showing phenotypic symptoms of hypothyroidism. This strain is thus primarily free from thyroiditis, but genetically related to the OS. The only obvious difference between the environmental conditions of the two groups of hens at Cornell was the increased room temperature for the OS. Upon arrival these eggs were incubated at 37·5°C and chicks derived from them were tested on the day of hatching for their 20-hr ^{131}I uptake (see Materials and Methods section for method).

Sequential study of ^{131}I uptake

Ten OS and five NWL chicks derived from hens that had been fed a normal laying diet (not supplemented with thyroxine or Protamone) for 4 weeks were used in this experiment.

Each bird was injected subcutaneously in the left leg with 1 μCi of ^{131}I (660,000 cpm) 2 days after hatching. They received water *ad libitum*, starting 7 hr after injection and starter corn feed 8 hr later. Use was made of a medical scintillation detector (Nuclear Chicago, model DS 8-1) with a 1-inch diameter collimator connected to an aural rate meter (Nuclear Chicago, model 1620 CS). Results were recorded graphically. At various time intervals the radioactivity of the thyroid was measured by placement of the detector directly over this region with the end of the probe touching the skin. Background radioactivity was estimated by recording the activity of the head region and this value was subtracted from that of the thyroid region. This method afforded valid measurements 4 hr or more after injection, but prior to this time the high level of radioactivity at the injection site prevented accurate thyroid uptake measurements. Correction was not made for radioactive decay.

Effect of thyroid antibodies on uptake of ^{131}I

The sera of ten 3-month-old OS chickens fed a normal growing feed were screened for thyroglobulin antibodies in passive haemagglutination tests using thyroglobulin-coated chicken red blood cells (chromic chloride method of Gold & Fudenberg, 1967). One bird with an antibody titre of 4000 was selected as a serum donor and sacrificed by heart puncture. To 15 ml of this serum was added 15 ml of saturated ammonium sulphate at 40°C and the resulting precipitate washed once with 50% saturated ammonium sulphate. This material was dissolved and dialysed against PBS for 3 days at 4°C. It was then cleared of remaining lipids and lipoproteins by centrifugation at 56,000 $\times g$ for 1 hr and sterilized by passage through a millipore filter (45 μm pore size). From the original 15 ml of serum we obtained 6 ml of a 2% protein solution. This had a passive haemagglutination titre of 4000 and a gel precipitation titre against 0·1% chicken thyroid extract of 2.

For control purposes normal chicken serum was fractionated in the same way, and in addition normal chicken gamma-globulin (Cohn FII, Miles Laboratories, Kankakee, Illinois) was also diluted in PBS and sterilized by use of a Millipore filter. Both these solutions had no detectable antibodies to thyroid antigens.

The antibody-containing or one of the two control preparations (0·2 ml) were then injected into a large chorioallantoic vein of 15-day-old NWL embryos purchased locally (for injection method see Beveridge & Burnet, 1946). On the day of hatching these chicks were injected with ^{131}I (660,000 cpm) subcutaneously. Twenty hours later the chicks were sacrificed by heart puncture, the blood was tested for the presence of haemagglutinating thyroglobulin antibodies and the thyroids were immediately frozen and their radioactive emission tested in the well-type gamma counter. These frozen glands were then further analysed in direct immunofluorescence (DIF) tests as described previously (Wick *et al.*, 1970). Briefly, 4 μm thick frozen sections of the two thyroid glands of each chicken were prepared, air-dried for 20 min and fixed in a solution of equal parts of 95% ethanol–ether for 10 min at room temperature. After a wash in PBS for 15 min the sections were treated with the gamma-globulin fraction of a FITC-labelled anti-chicken immunoglobulin rabbit serum. Control sections were similarly treated with an anti-human immunoglobulin conjugate of goat origin. Readings of slides were done without knowledge of their pretreatment on a Reichert–Zetopan microscope equipped with appropriate illumination and filters as previously described (Albini, Herzog & Wick, 1972).

The same gamma-globulin preparations were also injected i.p. into newly hatched NWL male chicks obtained locally (0·5 ml/chick; five chicks/group). After 18 hr ^{131}I (660,000 cpm)

was injected subcutaneously. The 7-hr thyroid uptake was then determined as previously described. The sera were tested for thyroglobulin antibody and the thyroids and kidneys studied by DIF for gamma-globulin deposits.

RESULTS

Protamone in diet replaced by pure T_4 and T_3

The aim of this experiment was to learn if the increased ^{131}I uptake exhibited by OS chickens in comparison to NWL controls, when both were derived from Protamone-fed hens (Sundick & Wick, 1974), could still be demonstrated after replacement of Protamone in the feed by T_4 and T_3. It can be seen that as long as 5 days after the feed switch (Fig. 1)

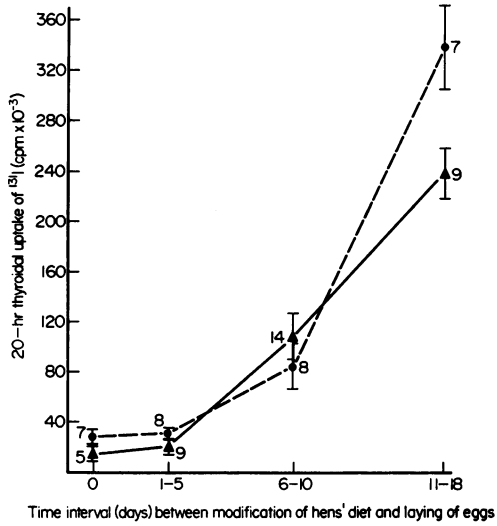

FIG. 1. Thyroid function of newly hatched chicks injected i.p. with ^{131}I (660,000 cpm) derived from eggs laid by OS (● – – ●) and NWL (▲ – – ▲) hens at various times after replacement of Protamone in their diet by pure T_4 and T_3. The numbers represent the number of chicks per group. Mean ±s.d. is given in each case.

no significant change occurred; the OS thyroid glands incorporated significantly more ^{131}I than the NWL controls, but these values were considerably below those previously obtained from offspring of non-Protamone-fed NWL hens. Between 6 and 10 days after the change the effects of Protamone were significantly diminished, but the uptake values within the two groups were so variable that no statistical significant difference was attained. Eggs collected 11–18 days after the food switch seemed to be completely free from the inhibitory effects of Protamone since both groups had very high degrees of uptake. The OS, however, incorporated significantly more radioactivity than the NWL ($P<0{\cdot}02$), thus reconfirming the difference already observed between OS and NWL derived from Protamone-fed hens. This experiment shows that the increased uptake of the OS is independent of any non-hormonal Protamone effects.

Another indication of this was provided by a study of the total iodine present in the eggs

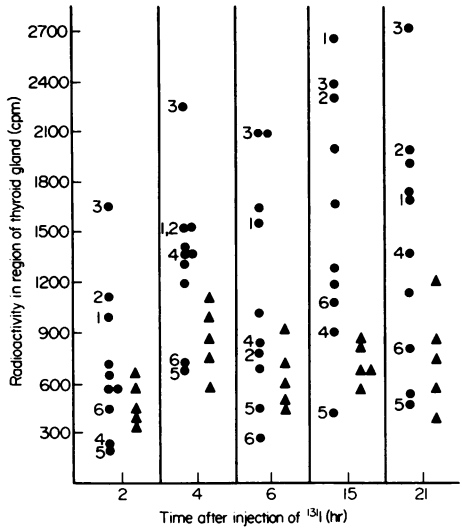

FIG. 2. Individual thyroidal ^{131}I uptake data for 2-day-old OS (●) and NWL (▲) chicks. Two-day-old OS and NWL chicks from non-Protamone fed hens were injected subcutaneously in the leg with 1 μCi of ^{131}I (660,000 cpm). At various time intervals the radioactivity of the thyroid region of the living chick was determined by use of a medical scintillation detector. Background radioactivity was estimated by recording the activity of the head region; this was subtracted from the thyroid region activity. Each point represents the uptake of one chick; the numbers identify individual chicks.

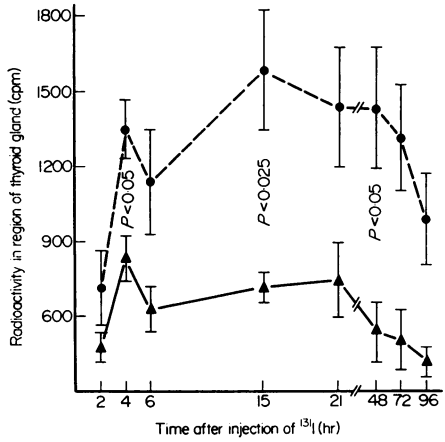

FIG. 3. Mean thyroidal ^{131}I uptake by OS (● – – ●) and NWL (▲ – – ▲) chicks following a single subcutaneous injection of 1 μCi of ^{131}I (660,000 cpm). At various time intervals the radioactivity of the thyroid region of the living chick was determined by the use of a medical scintillation detector. Background radioactivity was estimated by recording the activity of the head region; this was subtracted from the thyroid region activity. Ten OS and five NWL chicks were used in this study; the vertical lines represent the s.d. At 4, 15 and 48 hr the OS chicks incorporated significantly more ^{131}I than the NWL controls ($P<0{\cdot}05$).

laid 19–21 days after Protamone was replaced by pure T_4 and T_3. Eggs laid by the NWL hens contained $2{\cdot}4 \pm 0{\cdot}7$ μg of iodine/100 g of egg weight, while the OS had $4{\cdot}1 \pm 0{\cdot}2$ μg of iodine/100 g (five eggs/group; mean ±s.d.). Eggs laid by other OS hens continuously fed Protamone showed much higher levels of iodine ($18{\cdot}5 \pm 5{\cdot}8$ μg of iodine/100 g of egg).

Comparison of OS and Cornell (C) strain chicks

On the day of hatching nine OS and nine C strain chicks were injected i.p. with ^{131}I (660,000 cpm). Twenty hours later they were killed by ether inhalation and their thyroids removed. The OS thyroid glands had a mean radioactive emission value of $175{,}400 \pm 21{,}700$ cpm in comparison with a C strain value of $77{,}900 \pm 11{,}500$ cpm (mean ±s.d.). The increased uptake by the OS thyroid glands was highly significant by the Student's *t*-test ($P < 0{\cdot}005$). This difference was independent of the slight difference in body weight between the two strains (the OS were 3 g lighter), since comparison of only those OS and NWL of equal weight also resulted in a significant difference. Furthermore, any slight differences in the amount of non-radioactive iodide present in the chicks—which may have been the case, although it was not checked—would be unlikely to have influenced these results, since a simultaneous injection of potassium iodide ($K^{127}I$; 1 μg/chick) and ^{131}I had no significant effect on ^{131}I uptake (OS = $176{,}700 \pm 12{,}300$; C strain = $115{,}300 \pm 23{,}000$). The mixture and then injection of an even larger dose of $K^{127}I$ with ^{131}I (each chick receiving 8 μg of $K^{127}I$, i.e. approximately the same amount that each would have obtained from the egg of Protamone-fed hens assuming complete transfer of iodide from egg to chick) similarly failed to significantly affect the ^{131}I uptake of the OS ($145{,}400 \pm 29{,}500$) or the C strain ($68{,}400 \pm 23{,}800$) although both values showed a slight decrease.

Sequential study of ^{131}I uptake

In this study we examined the sequential thyroid uptake and release of ^{131}I by OS and NWL chicks after a single subcutaneous injection of ^{131}I (660,000 cpm). Fig. 2 illustrates the individual results of the ten OS and five NWL chicks from 2–21 hours after injection. It can be seen that the OS chicks exhibit a much wider range of uptake values than the NWL controls, some with values similar to those of the NWL (chickens 4, 5 and 6) and some with much higher uptakes (chickens 1, 2 and 3). It is interesting to note that the increased thyroid uptake of these latter chickens was already obvious 2 hr after the ^{131}I injection and remained so, with only minor exceptions, throughout the 21 hr listed in Fig. 2, and actually until the termination of the experiment 96 hr later.

The mean thyroid uptake of these OS and NWL chickens is shown in Fig. 3. In general, the difference between the two groups remained approximately constant throughout the experiment; at 4, 15 and 48 hr the differences were statistically significant ($P < 0{\cdot}05$). From the shapes of the two curves it appears that the increased amount of ^{131}I consistently found in the thyroid glands of OS chickens at 21 hr is due to the increased uptake of the isotope within the first few hr, and is not due to a decreased rate of release.

Effect of thyroid antibodies on uptake of ^{131}I

Since the possibility exists that the increased thyroidal uptake of ^{131}I by OS chicks may be due to maternally derived thyroglobulin antibodies, the eventual stimulatory effect of antibody-containing OS gamma-globulin on NWL chicks was examined.

The first step was to verify the presence of maternally derived antibody in our OS colony.

Nineteen newly hatched OS chicks were killed by heart puncture and the sera were tested by passive haemagglutinating for antibodies to thyroglobulin. Their mean $\log_2$ titre was 3·4± 0·7; while nine NWL controls were negative (no reaction at 1:2 dilution). The thyroid glands of five of the OS chicks were tested for gamma-globulin deposits by IF but none could be demonstrated. Next, 15-day-old NWL embryos were injected i.v. with 0·2 ml of either OS or normal chicken gamma-globulin. On the day of hatching they were injected with ^{131}I and their 20-hr thyroid uptake determined (Table 1). It can be seen that the thyroid uptake

TABLE 1. The effect of pretreatment with thyroglobulin antibodies on the thyroidal uptake of ^{131}I by newly hatched NWL chicks

Treatment	Number of chicks per group	Thyroid uptake ^{131}I (cpm)	Number with antibody per number tested	Number with gamma-globulin deposits in thyroid gland per number tested
OS gamma-globulin	9	135,000±24,000*	6/8	5/5
Normal gamma-globulin	10	99,300±13,400*	0/7	0/7
No gamma-globulin	8	131,600±22,500*	n.d.†	0/2

Fifteen-day-old NWL embryos were injected i.v. with 0·2 ml of either OS gamma-globulin (containing antibodies to thyroglobulin detectable by passive haemagglutination) or with normal chicken gamma-globulin. On the day of hatching these chickens and other untreated ones were injected with ^{131}I (660,000 cpm). Twenty hours later they were killed and their thyroids tested for radioactivity. Their sera were tested for thyroid antibodies by passive haemagglutination. The presence of gamma-globulin bound to the thyroid gland was determined by direct immunofluorescence.

* No significant difference by Student's *t*-test.
† N.d. = not determined.

of ^{131}I was not affected to a significant degree by the injection of OS gamma-globulin. However, the normal gamma-globulin appeared to result in a slight, albeit not significant, reduction. The sera of most recipients of OS gamma-globulin at the time of sacrifice contained detectable amounts of thyroglobulin antibodies, although the $\log_2$ titres were low, ranging between 1 and 4. An IF test on thyroid glands of these chickens revealed lumpy gamma-globulin deposits on the outer surface of the perifollicular basement membrane. Gamma-globulin was not found along the renal glomerular basement membranes. The thyroid glands

FIG. 4. Direct immunofluorescent staining of ethanol–ether fixed frozen sections of thyroid glands and kidney of newly hatched NWL chicks injected i.v. with 0·5 ml OS (= thyroglobulin antibody-containing) or normal chicken gamma-globulin 18 hr before sacrifice. All sections were treated with a FITC-labelled anti-chicken Ig conjugate. (a) Thyroid gland of a chick injected with normal gamma-globulin; no immunoglobulin deposits. (Magnification ×70.) (b) Granular immunoglobulin deposits along the follicular basement membrane in chick injected with OS gamma-globulin. (Magnification × 70.) (c) Granular immunoglobulin deposits along the follicular basement membrane in chick injected with OS gamma-globulin. (Magnification ×280.) (d) No immunoglobulin deposits along glomerular basement membrane in kidney of chick injected with OS gamma-globulin; double exposure, time. (Magnification ×70.)

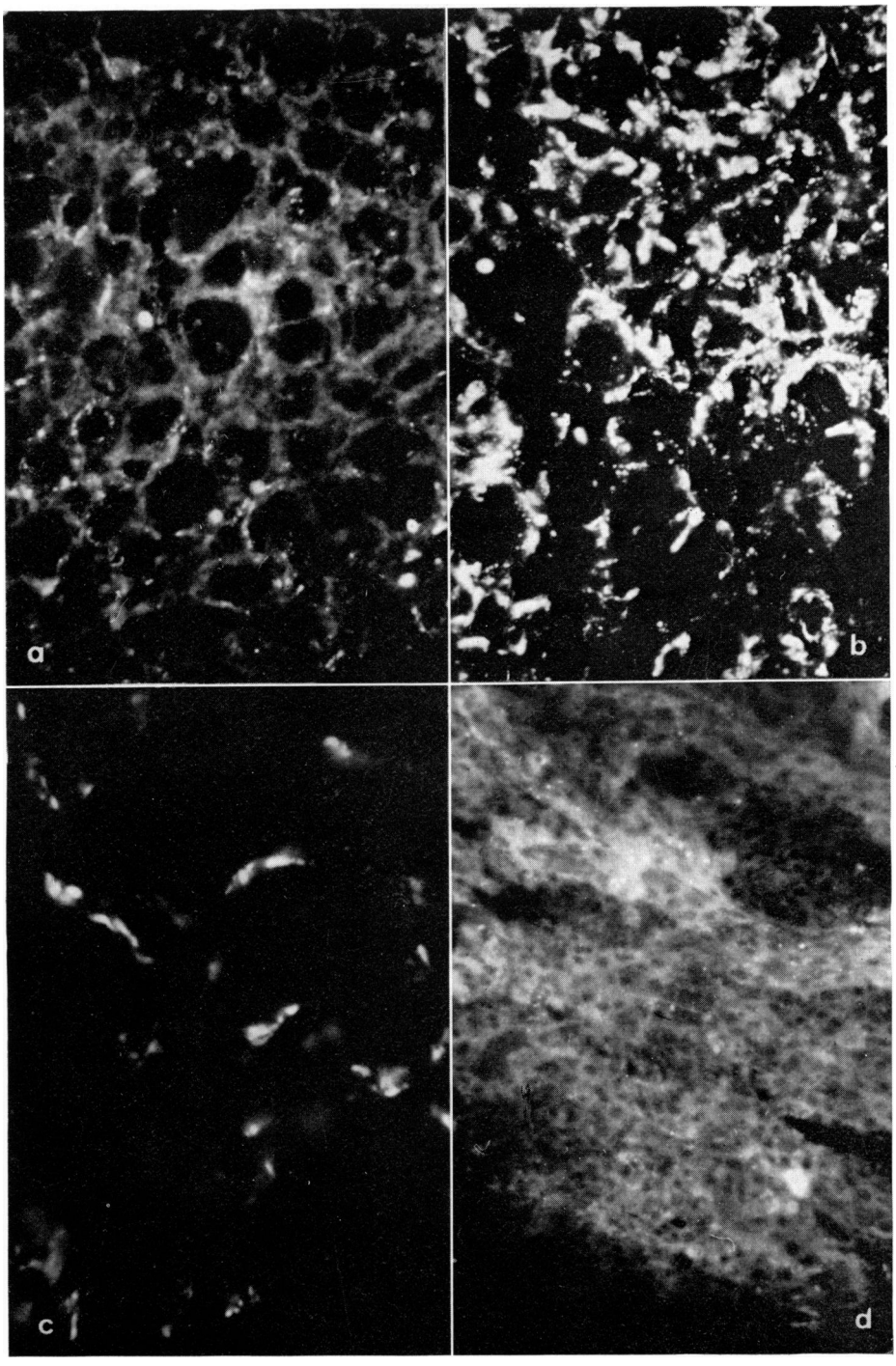

FIG. 4

of untreated or normal gamma-globulin treated NWL chicks contained no detectable gamma-globulin deposits.

The injection of newly hatched chicks with excessive amounts (0·5 ml) of OS or NWL gamma-globulin followed 18 hr later by a 7 hr ^{131}I uptake measurement yielded interesting results. The OS gamma-globulin-treated group had a significantly reduced uptake (132,000 ±30,000 cpm) when compared to those treated with normal gamma-globulin (232,000± 21,000 cpm; $P<0·025$). The thyroid glands of the former group had a striking amount of lumpy, bumpy deposits around the basement membrane (Fig. 4a, b, c). These were again not present along the renal glomerular basement membranes (Fig. 4d). The five recipients of OS gamma-globulin also had significant amounts of circulating thyroglobulin antibody 1 day after injection (mean $\log_2$ titre = 5·8), while recipients of NWL gamma-globulin showed no detectable titres.

DISCUSSION

All of the comparisons between OS and normal chicken strains reported here lead to the same conclusion: the thyroid glands of newly hatched OS chicks incorporate significantly more ^{131}I than appropriate controls under various conditions. These include: Protamone supplementation of the hens' diet, pure T_4 and T_3 supplementation or no dietary supplementation at all; the use of two different OS colonies and two normal chicken strains as controls—one being the strain from which the OS was developed—and the testing of uptake either after 20 hr, by sacrifice of the chicks and removal of the thyroids after injection, or sequential testing of the thyroid region of living chicks starting after 2 hr. In addition, uptake of ^{131}I by OS and NWL chicks in the presence of various amounts of non-radioactive ^{127}I also supported these findings.

The explanation for this is not known. The thyroids of newly hatched OS chicks appear normal by light microscopy. There is, therefore, no direct evidence that the immune system of the newly hatched OS chick stimulates the thyroid gland. The possibility does, however, exist that thyroglobulin antibodies or other thyroid antibodies—the former are the only ones known to be transferred from the hen to the egg (Kite *et al.*, 1969)—may stimulate the embryonic and neonatal chick thyroid gland, and these were detectable in the sera of our newly hatched OS chicks.

Stimulation of the thyroid by antibody could be effected in several ways. First, thyroxine-binding antibodies, which have been detected by Nilson, Rose & Witebsky (1971), in 16% of adult OS chickens, could conceivably be naturally transferred to the egg (this has not yet been demonstrated) and bind the thyroxine in the embryo and egg, thus stimulating the thyroid indirectly by a feed-back mechanism. Injection of OS sera containing thyroxine-binding antibodies into normal embryos did, in fact, stimulate the thyroid gland, as assessed by epithelial cell height. This, however, fails to explain the results of an experiment by Sundick & Wick (1974) in which 16-day-old OS and NWL embryonic thyroid glands were transplanted to the chorioallantoic membrane of 9-day-old NWL embryos (one OS and one NWL lobe/membrane). One week later the 20-hr ^{131}I uptake of both transplants was compared and the OS were found to incorporate significantly more. Since both glands were exposed to the same hormonal stimuli by the recipient for a week it appears unlikely to us that the OS gland would remain hyper-reactive due to thyroxine-binding antibodies.

A more likely factor that could explain the observed uptake by OS chicks is long-acting

thyroid stimulator (LATS) although it has not, to our knowledge, been detected in chickens. This has been found in the sera of approximately 50% of hyperthyroid patients and has been shown to be a class G immunoglobulin, probably directed against some constituents of the microsomal fraction of thyroid cells (Burke, 1967). It is believed to interact directly with the thyroid gland, and ferritin-labelled LATS has been located in the basement membrane region and the cytoplasm of thyroid follicular cells (El Kabir *et al.*, 1968). LATS has also been found in some Hashimoto patients and interestingly appears to induce thyrotoxicosis in the newborn children of some hyperthyroid mothers. Such a factor, if indeed present in OS chickens, could be responsible for the increased iodine uptake of the OS chicks, and even the OS thyroid transplants since, in contrast to thyroxine-binding antibodies, it binds to the gland itself. The inability to detect LATS isolated from patients in assays using newly hatched chicks (Lepp & Oliner, 1967) could be due to the lack of antigenic sites on chick thyroid cells cross-reacting for human LATS and it therefore does not exclude the existence of 'chicken' LATS.

The two antibody transfer experiments described in this report were primarily concerned with thyroglobulin antibodies due to their ease of detection, high incidence within the OS and their vertical transfer from the mother hen into the egg. In the first experiment embryonic NWL recipients of such antibodies were tested at hatching for their 20-hr ^{131}I uptake and no significant stimulation of thyroid uptake was observed. In the next experiment we therefore injected 2·5× more antibody and then tested thyroid uptake on the following day. This actually resulted in a significantly reduced radioiodide uptake, probably due to the large amount of gamma-globulin bound to the thyroid gland. It therefore seems unlikely that thyroglobulin antibodies lead to increased iodide uptake. However, it could not be excluded that an optimal amount or class of antibody injected into the unincubated egg might cause thyroid stimulation.

The comparison of OS and C strain chicks had two-fold significance. First, the OS hens used for this study laid eggs without ever having received Protamone or thyroxine. It is therefore likely that they were only mildly afflicted with thyroiditis, but their offspring nevertheless incorporated more ^{131}I than C strain controls. Second, the comparison with C strain was of particular importance, since all previous comparisons were between the OS and randomly bred white Leghorns obtained locally. These studies were subject to the criticism that differences in ^{131}I uptake could have been merely due to strain differences and unrelated to the etiology of thyroiditis. This is a reasonable criticism when one considers that Chai & Mellon (1972) developed by selective breeding two strains of mice, one with a high and the other with a low thyroidal iodine release rate, which was not related to autoimmune thyroiditis. However, selective breeding of C strain chickens resulted in the OS flock, and this latter strain has increased iodine uptake at the time of hatching and a severe autoimmune thyroiditis several weeks later. The similarity to Hashimoto's thyroiditis is striking, since it has been argued (Skillern *et al.*, 1956) that hyperthyroidism precedes thyroiditis. However, other authors (Nève, Ermans & Bastenie, 1972) dispute this finding.

A recent report by Newcomer (1973) provides some interesting information on thyroidal ^{131}I uptake by OS and C strain chicks from hatching up until several weeks of age. He measured the rate at which thyroidal radioiodide becomes protein-bound within a short period of time. However, he compared chicks derived from hens fed different diets (i.e. OS, but not C strain, hens were fed Protamone) and this probably explains the much reduced value of the OS from the 1st to the 4th day after hatching. At 3 weeks of age division of the

OS-chicks into those with severe and those with mild thyroiditis resulted in the expected finding that the former had almost no protein-bound radioiodine in the thyroid gland while the latter had an unexpectedly high value. The increased uptake rate by this latter group could have been due to increased pituitary stimulation of the slightly inflamed thyroid gland or to a genetic hyper-reactivity of the thyroid gland similar perhaps to that observed in our experiments on newly hatched OS chicks.

The comparison of offspring of OS and NWL hens after the diet switch from Protamone to pure T_4 and T_3 revealed the effect of Protamone in reducing thyroidal uptake of radioiodine. As suggested by Wheeler & Hoffman (1950) and Sundick & Wick (1974) this is not primarily due to a simple competition between ^{127}I and ^{131}I, but probably to an inhibitory effect of the iodinated protein compounds in Protamone that do not function as T_4 and T_3. This is further supported by the most recent studies which show that replacement of Protamone by T_4 and T_3 completely reversed this inhibition after 11 days of feeding. The comparisons between OS and Cornell C strain, in which 8 μg of $K^{127}I$ was mixed with the carrier-free ^{131}I, similarly indicated that ^{127}I does not cause a large reduction in uptake of ^{131}I, presumably because the thyroid adjusts to the increased amount of iodide in the circulation by proportionally increasing its uptake. When one considers the report by Tienhoven & Cole (1962) in which OS and C strain chicks fed Protamone had increased frequencies of thyroid abnormalities, it is important to consider that all future studies with OS chickens, particularly immunological and endocrinological, should be carried out using T_4 and/or T_3 rather than Protamone in the diet. The dose used in these experiments (based on data in the literature using normal chickens) resulted in reasonable egg production, but systematic dosage studies on the OS chickens might establish a more optimal level.

In future experiments it should be attempted to: (1) better characterize the established thyroid hyper-reactivity observed in OS chicks; and (2) to relate the observed abnormality with the subsequent development of thyroiditis. With regard to the former—studies of protein-bound iodine and butanol extractable and non-extractable iodine in the serum might indicate whether the increased uptake of iodide by the thyroid gland results in increased levels of circulating thyroxine or other non-biologically active iodinated proteins. The second problem could be approached by using the medical scintillation detector described in this report to record thyroidal radioiodine uptake of newly hatched OS chicks, then waiting several weeks to examine the thyroids for lymphoid infiltration.

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REFERENCES

ALBINI, B., HERZOG, F. & WICK, G. (1972) Photometric evaluation of indirect immunofluorescence chessboard titrations for the characterization of FITC-labelled antibodies. *Immunology*, **23**, 343.

BEVERIDGE, W.I.B. & BURNET, F.M. (1946) The cultivation of viruses and rickettsiae in the chick embryo. *Spec. Rep. Ser. med. Res. Coun. (Lond.)*, **256**.

BURKE, G. (1967) On the interaction of long-acting thyroid stimulator with thyroid microsomes. *J. clin. Endocr.* **27**, 1095.

CHAI, C.K. & MELLON, A. (1972) Selective breeding for variations in thyroidal iodine release rate in mice. *J. Endocr.* **55**, 233.

COLE, R.K., KITE, J.H. & WITEBSKY, E. (1968) Hereditary autoimmune thyroiditis in the fowl. *Science*, **160**, 1357.

DELESPESSE, G., BASTENIE, P.A., VANHAELST, L. & NÈVE, P. (1972) Thyroid autoimmunity. *Thyroiditis and Thyroid Function* (Ed. by P. A. Bastenie and A. M. Ermans), p. 39. Pergamon Press, Oxford.

EL KABIR, D.J., BENHAMOU-GLYNN, N., DONIACH, D. & ROITT, I.M. (1968) The localization of ferritin-labelled thyroid stimulating globulin (LATS) in the follicular cell of the thyroid of the mouse. *Proceedings of the Third International Congress of Endocrinology, Excerpta medica. Foundation, International Congress Series*, **157**, abstract 226.

FALCONER, I.R. (1971) The thyroid glands. *Physiology and Biochemistry of the Domestic Fowl* (Ed. by D. J. Bell and B. M. Freeman), p. 459. Academic Press, London.

GOLD, E.R. & FUDENBERG, H. (1967) Chromic chloride: a coupling reagent for passive hemagglutination reactions. *J. Immunol.* **99**, 859.

KITE, J.H., FLANAGAN, T.D., SUNDICK, R.S. & WITEBSKY, E. (1969) Factors associated with the development of spontaneous autoimmune thyroiditis in chickens. *Fed. Proc.* **28**, 631.

LEPP, A. & OLINER, L. (1967) Failure of long-acting thyroid stimulator globulin (LATS) and serum to stimulate thyroid function in the chick. *Endocrinology*, **80**, 369.

NÈVE, P., ERMANS, A.M. & BASTENIE, P.A. (1972) Struma lymphomatosa (Hashimoto). *Thyroiditis and Thyroid Function* (Ed. by P. A. Bastenie and A. M. Ermans), p. 109. Pergamon Press, Oxford.

NEWCOMER, W.S. (1973) Accumulation of radioiodine in thyroids of chicks of the Obese strain with hereditary thyroiditis and of their parental strain. *Gen. comp. Endocr.* **21**, 322.

NILSSON, L.A., ROSE, N.R. & WITEBSKY, E. (1971) Spontaneous thyroiditis in the Obese strain of chickens. VI. Thyroxine-binding antibodies. *J. Immunol.* **107**, 997.

RAHEJA, K.L. & SNEDECOR, J.G. (1971) Some effects of single doses of triiodothyronine and thyroxine in hypothyroid chicks. *Gen. comp.* Endocr. **16**, 97.

SKILLERN, P.G., CRILE, S., JR, MCCULLAGH, E.P., HAZARD, J.B., LEWIS, L.A. & BROWN, H. (1956) Struma lymphomatosa: primary thyroid failure with compensatory thyroid enlargement. *J. clin. Endocr.* **16**, 35.

SUNDICK, R.S. & WICK, G. (1974) Thyroid function of embryos and newly hatched chicks derived from Obese strain (OS) chickens with spontaneous autoimmune thyroiditis. (Submitted for publication.)

TIENHOVEN, A. VAN & COLE, R.K. (1962) Endocrine disturbances in obese chickens. *Anat. Rec.* **142**, 111.

VANHAELST, L., BONNYNS, M., ERMANS, A.M. & BASTENIE, P.A. (1972) Heredity of autoimmune thyroiditis. *Thyroiditis and Thyroid Function* (Ed. by P. A. Bastenie and A. M. Ermans), p. 303. Pergamon Press, Oxford.

WHEELER, R.S. & HOFFMAN, E. (1950) The etiology of goitre in chicks from thyro-protein-fed hens; negative role of inorganic iodide. *Poultry Sci.* **29**, 306.

WICK, G., SUNDICK, R.S. & ALBINI, B. (1974) Review. The Obese strain (OS) of chickens: an animal model with spontaneous autoimmune thyroiditis. *Clin. exp. Immunopathol.* (In press.)

WICK, G., WITEBSKY, E., KITE, J.H., JR & BEUTNER, E. (1970) Immunofluorescent studies of thyroid autoantibodies in chickens of the Obese strain (OS). *Clin. exp. Immunol.* **7**, 173.

WITEBSKY, E., KITE, J.H., WICK, G. & COLE, R.K. (1969) Spontaneous thyroiditis in the Obese strain of chickens. I. Demonstration of circulating autoantibodies. *J. Immunol.* **103**, 708.