the differences in various subjects' levels in two ways. First, the individual with a low immunoglobulin level might have fewer antibody classes than the person with a high level. This would seem to be the result of one person being exposed to less antigens than another—an environmental effect. Alternatively, the individual with low levels may have the same number of antibody classes, but fewer molecules in each class. This would imply a difference among individuals in capacity to respond to the same antigens. This would seem to be an effect of heredity on immunoglobulin levels and could be tested.

Summary. Immunoglobulin levels in 15 individuals were followed weekly for 25 weeks. The average variation was ± 17% at 2 standard deviations. This is small compared to the variation found among individuals; therefore the level of serum immunoglobulins within an individual is relatively stable.

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Effect of Melatonin upon Thyroid Hormone Secretion Rate and Endocrine Glands of Chicks.*† (32105)

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Recently Wurtman *et al*(1) suggested that the pineal is an endocrine gland and secretes the hormone melatonin which influences the immature female reproductive system of the rat. The ability of the rat pineal gland to synthesize melatonin was reported to be markedly reduced by exposure to light(2) and enhanced by constant darkness(3,4). It would thus appear that melatonin depresses the development of the ovary, and light, by depressing melatonin secretion, stimulates the gonadotropic hormones and ovarian development.

While it has long been known that increasing light stimulates the reproductive cycles of seasonal breeding birds(5), ducks(6) and precocious egg production in pullets(7), it was suggested by these investigators that light influences gonad development *via* the eye and the hypothalamus. The observation on the rat suggests that the pineal gland is the receptor of light and darkness stimuli, and the alteration in melatonin secretion is the mechanism by which sexual maturity is influenced.

In a study of the effect of pinealectomy and pineal injections(8) in 20-day-old male White Leghorns, it was reported that pinealectomy resulted in inhibition of testis growth, and pineal material increased testis growth in normal chicks and restored testis weight to normal in pinealectomized chicks. However, in a later study(9) in 40- to 65-day-old male chicks, it was reported that pinealectomy caused hypertrophy of the testes. The effect was reversed by the administration of pineal material to normal chicks.

In a histological study of the pineal body of normal birds (14 hours of light) and those maintained in darkness, it was observed that the parenchymal cells showed no change in morphology or staining behavior when in total darkness; however, the large epithelioid cells of the septum showed no magenta colored

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sonic activity on splenic and aveolar phagocytosis of particulate materials.

Experimental methods. Male Holtzman rats (250-300 g) maintained on Purina Laboratory Chow and tap water *ad libitum*, were used in all experiments. Rats employed as tissue donors were anesthetized with ether and rapidly desanguinated prior to liver, lung, and spleen removal. All organs were rapidly chilled in cold isotonic saline and sliced with a Stadie-Riggs tissue slicer according to previously described procedures(14). All slices were washed and briefly maintained in cold isotonic saline prior to incubation.

The incubation media consisted of heparinized rat serum or heparinized Krebs-Ringer phosphate adjusted to a pH of 7.4. Heparin (Upjohn, Kalamazoo, Mich.) was employed at a concentration of 50 USP units per ml of incubation medium. Serum used as the incubation medium was obtained from normal, non-fasted rats by cardiac puncture. When necessary, serum dilution was accomplished with the Krebs-Ringer phosphate buffer.

Radio-iodinated triolein labeled "Re Test Lipid Emulsion"(15) stabilized in a 0.1% gelatin (Nutritional Biochem. Corp., Cleveland, Ohio) supplemented sterile dextrose (5%) and water solution was employed as the test particle. The emulsion base consisted of glycerol, I^{131}-triolein (Trioleotope, Squibb Laboratories), and alcohol soluble soybean lecithin in a ratio of 10:10:1 by weight, respectively(15). A dose of 2000 μg of I^{131}-labeled triglyceride emulsion was added to each incubation vial.

Incubation samples which consisted of 3 ml of heparinized incubation medium, gelatin supplemented I^{131}-"RE Test Lipid Emulsion," and either liver, lung, or spleen tissue slices were gassed with 95% O_2 and 5% CO_2 prior to incubation in a Dubnoff metabolic shaker at 37°C for 30 minutes. Following the incubation procedure all tissues were removed, washed, and analyzed for total tissue radioactivity. Accumulated hepatic, splenic, and pulmonary tissue radioactivity was determined with a Nuclear-Chicago crystal scintillation system. Data are expressed as the per cent of added radioactivity (% I.D.) phagocytized per 100 mg of wet weight of tissue or the μg of triglyceride phagocytized per 100 mg of tissue.

TABLE I. Hepatic* Phagocytosis of Gelatinized "RE Test Lipid Emulsion" as a Function of Serum† Concentration.

% Serum in incubation medium	No. of incubated samples	% ID‡/100 mg tissue (mean ± SE mean)	% Maximum uptake§
.0	11	.74 ± .16	4.7
5.0	4	1.87 ± .19	11.9
10.0	4	2.70 ± .25	17.2
16.7	11	9.65 ± 1.41	61.6
25.0	4	11.65 ± .49	74.4
33.3	8	15.72 ± 2.18	100.4
66.7	7	15.11 ± 1.82	96.5
100.0	7	15.66 ± 3.31	100.0

* Liver tissue was obtained from 6 rats.
† Serum was pooled from 16 rats.
‡ Injected dose (ID) equaled 2000 μg of I^{131}-labeled triolein lipid emulsion.
§ Maximum uptake was equivalent to that observed in 100% serum medium.

Results. In an attempt to demonstrate the effect of opsonization on *in vitro* phagocytosis, hepatic uptake of the gelatinized I^{131}-"RE Test Lipid Emulsion" is presented as a function of the serum concentration in the incubation medium (Table I). Kupffer cell phagocytosis of the lipid particles in the heparinized Krebs-Ringer phosphate medium without serum was 0.74% of the injected dose per 100 mg of hepatic tissue. In marked contrast to such minimal uptake, the addition of serum to the incubation medium produced a significant enhancement of phagocytosis with a maximal phagocytic uptake equivalent to 15.72% of the injected dose being observed when serum was used in a concentration of 33.3% (Table I). Further increases in serum concentration, *i.e.*, up to 66.66 and 100% had no effect on the degree of hepatic phagocytosis.

The comparative evaluation of hepatic, splenic and pulmonary phagocytic activity is presented in Table II. In agreement with previously reported observations(15), as well as those presented in Table I, hepatic phagocytosis was minimal in Krebs-Ringer phosphate and markedly enhanced by the presence of heparinized serum. The use of heparinized serum as the incubation medium resulted in a comparable enhancement in splenic phagocytosis as indicated by the 38-

TABLE I. Effect of Melatonin Upon Weights of Certain Endocrine Glands of Chicks at 10 Weeks of Age.

No. of chicken	Mean b.w. at 10 wks of age g	Mean testes or ovary wt		% of increase or decrease from control	Mean adrenal wt		% of increase or decrease from control	Mean thymus wt		% of increase or decrease from control
		Total ± S.E. g	g./100 g. b.w.		Total ± S.E. mg	mg/100 g. b.w.		Total ± S.E. g	g./100 g. b.w.	
Group I										
Male (14)	2266.8	18.42 ± 2.75[a]	.81		367.14 ± 29.20[g]	16.20		9.26 ± 1.06[m]	.41	
Female (4)	1804.7	2.63 ± .8[b]	.14		207.5 ± 13.76[h]	11.50		5.71 ± 1.64[n]	.32	
Group II (treatment 50* μg./100 g. b.w.)										
Male (10)	2379.0	9.48 ± 3.01[c]	.40	—48.53	300.0 ± 27.59[i]	12.61	—18.29	8.80 ± 1.41[o]	.37	— 4.97
Female (8)	2026.0	1.74 ± .28[d]	.09	—33.84	206.25 ± 38.16[j]	10.18	— .60	7.09 ± .97[p]	.35	+24.17
Group III (treatment 100 μg.*/g. b.w.)										
Male (8)	2315.4	1.13 ± .15[e]	.05	—93.84	253.37 ± 15.69[k]	10.94	—30.09	2.51 ± .64[q]	.11	—72.90
Female (11)	1923.3	.86 ± .06[f]	.04	—67.30	135.0 ± 11.09[l]	7.02	—34.94	2.54 ± .53[r]	.13	—55.52

No. of chicken	Mean b.w. at 10 wks of age g	Mean thyroid wt		% of increase or decrease from control	Mean pituitary wt		% of increase or decrease from control	Mean pineal wt		% of increase or decrease from control
		Total ± S.E. mg	mg/100 g. b.w.		Total ± S.E. mg	mg/100 g. b.w.		Total ± S.E. mg	mg/100 g. b.w.	
Group I										
Male (14)	2266.8	292.04 ± 50.88[s]	12.90		22.4 ± .68	.99		5.89 ± .74	.26	
Female (4)	1804.7	274.5 ± 13.14	15.21		17.1 ± .58[u]	.95		5.60 ± 1.67	.31	
Group II (treatment 50* μg./100 g. b.w.)										
Male (10)	2379.0	392.6 ± 53.[t]	16.50	+34.43	20.66 ± 2.75	.87	— 7.71	6.44 ± .67	.27	+ 9.34
Female (8)	2026.0	270.55 ± 21.73	12.26	— 1.74	24.48 ± 3.72[v]	1.11	+43.1	7.33 ± .96[x]	.33	+30.89
Group III (treatment 100 μg.*/g. b.w.)										
Male (8)	2315.4	296.5 ± 37.28	12.81	+ 1.02	22.97 ± 4.43	.99	+ 2.54	7.25 ± 1.29	.31	+28.09
Female (11)	1923.3	280.85 ± 30.78	14.60	+ 2.31	28.22 ± 6.23[w]	1.47	+65.03	4.86 ± .67[y]	.25	—13.29

S.E. = Standard error
g. b.w. = Gram body wt
Student "t" test:
* Melatonin

a vs. c $P<.05$; a vs. e $P<.001$; c vs. e $P<.01$; b vs. f $P<.025$

b vs. d $P<.20$; g vs. i $P<.10$; g vs. k $P<.005$; i vs. k $P<.10$

h vs. l $P<.001$; j vs. l $P<.05$; m vs. q $P<.001$; n vs. r $P<.05$

o vs. q $P<.001$; p vs. r $P<.001$; s vs. t $P<.10$; u vs. v $P<.05$

u vs. w $P<.05$; x vs. y $P<.05$

were unaffected but in the females, there was an increase of 65% (P<.05).

The pineal glands of both sexes were increased on the lower level of melatonin but at the higher level, the males showed a 23% increase while the females showed a 13% decrease in weight.

Discussion. Kitay and Altschule(12) have reviewed the earlier literature concerning the physiological role of the pineal gland in fowls, to which the reader is referred. With the recent isolation of melatonin and the suggestion that it is a hormone of the pineal gland, there has been a resurgence of interest in this gland and its hormone. That the secretion of this "hormone" is influenced by light (or darkness) suggests that the pineal glands may be the receptor of light stimulation in relation to seasonal breeding animals.

Most of the recent research has been concerned with studies using rats as the experimental animals. Since it has been recognized for a long time that increasing light stimulates precocious egg production, it seemed desirable to determine the effect of melatonin on the weights of the various endocrine glands and concurrently determine the effect upon TSR.

In unpublished research from this laboratory it has been shown that administration of melatonin, at levels comparable to those given to the chickens in the present experiment, significantly depressed TSR in both rats and hamsters. No explanation can be offered to explain the difference between mammals and birds.

It has been shown in rats that melatonin significantly depresses gonad weight. The present study indicates that melatonin has a similar effect in immature birds. It has been shown that pinealectomy in 40 to 64 day-old cockerels caused testes hypertrophy and pineal extracts caused the reverse(9). To this extent, the absence and presence of melatonin is comparable in rats and chickens. The only study which is difficult to harmonize is the report that in fowls the enzyme stimulating melatonin secretion (HIOMT) was highly significantly decreased in darkness compared to those in constant light(11). If melatonin secretion is actually depressed in darkness and stimulated by constant light in chicks (the reverse of that in rats) then melatonin should not have depressed gonad weight which was observed.

In the present study, the weights of both the adrenals and thymus glands were significantly depressed. Whether this depression plays a role in gonad function or is an independent action of melatonin on the secretion of ACTH by the pituitary will require further study.

Summary. The normal TSR of a group of 55 male and female chicks was determined starting at 4 weeks of age. They were then divided into 3 groups of equal TSR. The mean TSR of a control group and groups injected with 50 and 100 μg of melatonin/100 g bw/day determined starting at 7 weeks were approximately equal, indicating that melatonin had no effect upon the TSR of immature chickens. However, at 10 weeks of age melatonin significantly reduced the testes weights 94% (P<.001) and ovaries 67% (P<.025) at the higher level as well as the adrenal and thymus glands. The pituitary gland weights of the females were progressively increased on the two levels of melatonin while the males were unaffected. The pineal glands were increased in both sexes at the lower level. At the higher level, the males showed an increase but the females showed a slight decrease.

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Effect of Intestinal Contents on Uptake of Radioiron by Everted Rat Gut Sacs.* (32106)

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Although much attention has recently been directed to the effect of gastric(3) and pancreatic(5) secretions on the absorption of iron, the role of intestinal contents has been neglected. Iron absorption can take place along the whole gastrointestinal tract (8) and even the colon can absorb ferrous iron(7). It seems likely that when there is an increased demand for iron these potential sites of absorption may play an important part. If this is so, then intestinal intraluminal factors may also be important in this process. During the investigation of the uptake of Fe^{59} by everted intestinal sacs obtained from rats, we noticed that sacs prepared from feeding rats ("unfasted") took up more isotope than sacs from fasted rats. We decided to investigate further the effect of intestinal contents and other materials on the *in vitro* uptake of Fe^{59} by everted gut sacs.

Method. The gut sacs were prepared and used according to the method we have already described(6), using the first 20 cm of gut beyond the pylorus to provide 4 segments of 4 cm from each rat. At least 4 such sequential segments from one or more rats were used to study a single test material. The segments were first exposed to the prepared secretion or test material at 37°C for 30 minutes, then washed in ice cold phosphate buffer at pH 7.2. The uptake of Fe^{59} by the segments as ferrous citrate was then determined and recorded as an index of uptake(6). The secretions tested and their methods of preparation were as follows:

(1) Unfasted gut wash consisted of the contents of the first 60 cm of gut beyond the stomach washed into 5 ml of buffer, homogenized in a Waring blendor and neutralized with 5% sodium bicarbonate.

(2) Fasted gut wash was prepared as in (1) from rats fasted 18 hours.

(3) Fasted stomach contents were obtained by emptying the stomach into 5 ml of buffer and treating as in (2).

(4) Unfasted stomach contents were obtained and prepared as in (1).

(5) Histamine stimulated stomach contents were obtained by injecting 1 mg of histamine intravenously into fasting rats and collecting the secretions as in (1).

(6) Fasted rats were given 0.245 units of pancreozymin, 0.24 units of secretin and 1 mg of histamine intravenously and the gut contents collected as in (5).

(7) Five grams of Laboratory Chow were homogenized with buffer, filtered through gauze and neutralized with sodium bicarbonate.

(8) Ascorbic acid 1 mg was added to 7 ml of buffer and neutralized with sodium bicarbonate.

(9) Unfasted gut washed was prepared as in (1), and then boiled for 30 minutes.

All intestinal and gastric contents were adjusted to pH 7.2 to avoid the effect of varying pH. Sixty fasted Sprague-Dawley rats weighing approximately 225 g and with normal hemoglobins were used to provide 240 gut sacs. Segments from "unfasted" rats were also used as further controls.

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