of UC should be considered, but do not explain this finding. Liver disease is frequent in patients with UC, but we have found an increased titer of capsular agglutinating activity in sera from patients with Laennec's cirrhosis. Protein loss into the intestinal tract occurs in UC, but our patients had normal agglutination titers against 2 of the 3 *E. coli* strains studied. This would be unusual in protein-losing enteropathy, which is not characterized by selective antibody loss (8). The observation that lymphocytes taken from patients with active UC fail to react in the presence of the K antigen of O 119:B 14 *in vitro*(9) suggests that our findings are due to decreased production of agglutinating antibodies to this antigen.

The low agglutinin titer against the capsular antigens of known pathogens such as *E. coli* O 119:B 14(10) may increase direct damage to the UC colon by these organisms. Alternatively, bacterial invasion could furnish a repeated antigenic stimulus leading to further mucosal damage by an autoimmune mechanism(11). In this context, our finding that UC and normal sera have similar agglutinating activity against *E. coli* O 14, reported to have immunologic similarity to sterile human fetal colon antigen, is of interest(12). It should be notel that we did not study specific components of *E. coli* O 14, such as the common antigen of Kunin(13).

Summary. Sera from patients with chronic ulcerative colitis have low agglutination titers against a pathogenic strain of *E. coli,* due wholly or in part to a decreased content of 19S antibodies.

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Stability of Human Immunoglobulin Levels.* (32104)

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Immunoglobulin levels vary widely among individuals, but the variation within the individual has not been studied. This information would be useful in understanding immunoglobulin metabolism and in evaluation of patients on whom repeated levels have been obtained. This study reports the amount of variation observed in the immunoglobulin levels of 15 healthy subjects who were bled weekly for 6 months. The results show that levels in the average individual vary within ± 17% (2 standard deviations) of their estimated mean and are, therefore, quite stable.

Materials and methods. Subjects. Seven men and 8 women between the ages of 20 and 35 were bled once a week from a finger stick for 25 consecutive weeks. At the time of bleeding each reported on his general health, time of last meal, any medication

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taken, and any unusual stress, psychological or physical.

Methods. IgG, IgA, and IgM were quantitated by a single radial immunodiffusion method(1) using commercially available antibody-agar plates (Immunoplates, Hyland Laboratories, Los Angeles, Calif.). The plates were standardized against purified immunoglobulins, each of which, in Ouchterlony analysis, gave one precipitin line when allowed to diffuse against an antiserum to whole human serum. The protein concentrations of the purified preparations were determined by micro-Kjeldahl analysis. Standardization curves used were the least-squares linear regressions of the log mg per 100 cc on the log ring diameter. A full logarithmic relationship was chosen instead of the semi-logarithmic one used by others(2-4) since this form fits the data better.

Incubation times and temperatures used for the antibody-agar plates were 4 hours ± 5 minutes at 37°C for IgG and 16 hours ± 1 hour at room temperature for IgA and IgM.

Controls. Since the variation of the subjects' levels was in the same range as variation of the technique, particular attention was paid to obtaining an accurate estimate of the error of the technique.

Two secondary standards of human sera were used in each antibody-agar plate. Any plate in which the controls varied more than two ring diameters from the expected was discarded. The standards were formed from single bleedings of 300 cc of 2 normal individuals. Small volumes of serum (0.2 ml) were preserved frozen at —70°C until the day of use. Neither the primary nor the secondary standards deteriorated over a one-year period. All values for one of the secondary standards were accumulated over the 25-week period of the study giving 126 values for IgG, 128 values for IgA, and 64 values for IgM. Fewer control values were accumulated for IgM since twice as many determinations per plate were possible for this immunoglobulin.

To evaluate whether the error of the technique based on many values from one day varied significantly from the error based on values from several days, an F test for block effects was performed comparing the variance of the means for different days to the variance of values for any one day. All computations were performed on the log of mg per 100 cc. The F value was largest for IgG (4.11, $p<.005$). This high F value, as compared to that for the other globulins, stems from our using the same standardization line for the first 6 IgG plate lots. On the basis of multiple determinations on several antigen concentrations for each new lot, we had concluded the lots were the same. In retrospect, they differed slightly. The F values for IgA and IgM were 1.48 ($p<.1$) and 1.94 ($p<.05$) respectively. This indicates that the error of the technique over a period of time is probably larger than that for any given day and this should be taken into account when the amount of error is important to the conclusions of the study.

To estimate the error of the technique for this study, it was considered appropriate to take one control value from the same secondary standard for each day of the study period on which immunoglobulin determinations were made. Statistics for the error were performed on 32 values for IgG, 46 for IgA, and 29 for IgM. At 2 standard deviations from the mean, IgG varied ± 12%; IgA ± 10%; and IgM ± 14%. The error of the technique for all 3 globulins was taken as ± 12%.

Statistical methods. Immunoglobulin values from normal individuals are distributed in a log normal fashion, as determined from plots on probability paper, hence computations of averages and standard deviations were performed on the log of the mg per 100 cc. After computation, values were retranslated into mg per 100 cc. With this conversion, the 2 standard deviations in the negative direction were less than the 2 standard deviations in the positive direction. As this difference was small in the subjects, the two deviations were averaged. The differences between the negative and positive deviations in the adults were large and the separate values are given in Table I. These large differences reflect the wide range over which values from different individuals can occur.

Table I. Variation of Immunoglobulin Levels of 15 Subjects Studied Weekly for 25 Weeks.

Subject	Age	Sex	IgG Mean	IgG % Variation	IgA Mean	IgA % Variation	IgM Mean	IgM % Variation
1	27	M	965	±18*	49	±21	48	±32
2	31	M	1126	±15	231	±13	77	±23
3	31	M	971	±17	124	±13	94	±16
4	25	M	1050	±13	246	±14	126	±21
5	29	M	1348	±11	407	±14	169	±18
6	27	M	1261	±14	196	±15	133	±19
7	30	M	1399	±13	247	±22	121	±29
8	25	F	975	±14	178	±10	132	±17
9	25	F	1212	±13	80	±18	137	±19
10	35	F	993	±14	309	±18	105	±17
11	23	F	1054	±17	198	±26	72	±26
12	24	F	878	±15	105	±18	186	±20
13	20	F	1004	±18	301	±15	115	±23
14	25	F	1501	± 8	284	±18	112	±23
15	25	F	1162	±15	179	±14	203	±18
Avg. Variation				±14		±17		±21
Control			1441	±12	282	±10	141	±14
Normal Adults (n=315)			1045	−32 +47	169	−64 +189	89	−58 +138

* ±2 standard deviations in percent of mean.

Results. The average variation (± 2 SD) of immunoglobulin levels was ± 17% and ranged from ± 8% in subject no. 14 to ± 32% in subject no. 1. The observed variation for each individual's IgG, IgA, and IgM is listed in Table I. As the deviations of the subjects' levels did not seem to be different from those of the controls, further analyses, such as correlating variation in one class with that in the other classes, etc., were not justified.

Fourteen of the 15 subjects had at least one upper respiratory tract infection during the study and several had two or three. Almost every member took a variety of medications including aspirin, various types of antihistamines, a few tranquilizers, and 2 took oral contraceptive medications. Other situations that arose were a primary reaction to smallpox vaccination, injections of .01 cc per kilo of gamma globulin in week 6 for subjects no. 1, 3, and 8 for exposure to infectious hepatitis, a voluntary 22 lb weight loss in one subject, very irregular eating habits in another subject, and various psychological stresses and strains (academic examinations, lack of sleep, etc.). During the study period 4 of the subjects suffered from hayfever. None of the above situations caused detectable changes in any of the levels.

A sixteenth individual, a 23-year-old male, was also studied. He contracted infectious mononucleosis, with enormous increases of all 3 immunoglobulin classes, and was excluded from the study. The nature and character of the immunoglobulin changes are being studied.

Discussion. Repeated measurements of immunoglobulin levels in healthy individuals vary no more than about 20%. The true variation is probably considerably less since the variation of the technique is about the same as that for the subjects.

At least 80% of the average number of immunoglobulin molecules are present at any one time. Since most, if not all, of the immunoglobulin molecules are probably antibody and continued antibody synthesis depends on antigenic stimulation, it is logical to assume that a fairly constant antigenic stimulus might be present. An alternate assumption would be that the antigen load is changing and feedback mechanisms allow expansion or contraction of antibody classes as needed to keep the level stable.

An individual's immunoglobulin level is characteristic of him. One might explain

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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