experiments the band containing infectious virus was harvested by side puncture and tested undilute for antigenic activity.

Sera used to detect antigens were obtained from 3 sources: a) hyperimmune rabbit sera which were prepared by immunizing rabbits with virus grown in rabbit kidney cells[7], b) sera obtained from rabbits which developed encephalitis after immunization by scarifying the eye and dropping 0.1 ml of crude virus into the scarified area 6–10 days prior to bleeding and c) outdated human plasma obtained from our blood bank. Microtiter assays were used for detection of antigen and virus activity and included complement fixation (CF), passive hemagglutination, and infectivity procedures[1,7,8].

Results and discussion. Initial experiments were concerned with separation of the infectivity from the soluble antigens to determine whether the antigenic activity detected by passive hemagglutination was primarily associated with either the infectious virus or the soluble antigens. When the viral preparations were separated by sucrose rate zonal centrifugation, the antigenic activity was found exclusively on the top of the gradient with little separation of the PHA and CF activity. The infectivity was found considerably lower in the gradient and separate from the antigenic activity. Additional attempts were made to separate the CF from the PHA activity using longer centrifugations in sucrose density gradients. In these studies the infectious virus was removed by high speed centrifugation prior to separation on sucrose gradients and the supernatant was then centrifuged for 17 h at $100{,}000 \times g$. Little separation of the antigenic activity was accomplished.

Inability to separate the PHA from the CF activity on sucrose necessitated the use of equilibrium centrifugation in CsCl. After infectious virus was removed as described above, the complement-fixing activity was readily separable from the PHA activity, indicating that these tests apparently measure different antigenic moieties. The PHA reactive antigen was less buoyant than the antigen detectable by complement fixation methods. Some difficulty was occasionally encountered in localization of CF activity into a single peak, which probably indicates heterogeneity of CF antigens.

During these studies, interest was also directed to the host response to the soluble antigens. In the above data, various antisera reacted with the antigens and gave similar results. More definitive studies were initiated by reacting antisera with various antigen preparations in order to determine whether or not the test sera had identical specificities to the various antigen preparations tested. Approximately 30 human plasmas were collected and tested by both PHA and CF methods. It was noted that complete agreement between those 2 methods was obtained: all of the plasmas positive by 1 method were also positive by the other with good correlation between titers. The Table shows the results of a typical experiment. Included in the antigens tested were 2 PHA antigens and 1 CF antigen all of which were separated in CsCl gradients, an antigen pool remaining on the surface of a sucrose gradient and the opalescent band containing infectious virus. It can be seen that some variation in PHA activity was apparent between these sera; however, it was minimal. It also should be noted that in this experiment the concentrated virus band was positive in the CF test but negative in the PHA test. It was subsequently found that concentrated virus was reactive in the PHA test when used undilute or at low dilution (1:2). It was not determined if the PHA activity of the virus was a result of available reactive sites on the intact virion or viral degradation which uncovered active sites.

Antibody titers[a] to *Herpes virus hominis* soluble antigens

Antigen	Rabbit hyperimmune		Rabbit acute infection		Human	
	CF[b]	PHA[c]	CF	PHA	CF	PHA
G4F 6–7[d]	<10	<40	<10	640	<10	10,240
G5F 8–10[d]	20	640	<10	160	10	10,240
G5F 13–14[e]	40	<40	<10	<40	20	<40
SG5 B[f]	320	<40	160	<40	160	<40
SG8 T[g]	320	10,240	160	1280	80	20,480

[a] Expressed as reciprocal of dilution. [b] Complement fixation. [c] Passive hemagglutination. [d] PHA positive formalin treated antigens separated on CsCl gradients. [e] CF positive formalin treated antigens separated on CsCl gradient. [f] Virus 'band' separated by sucrose gradient. [g] Soluble antigens on top of sucrose gradient.

Zusammenfassung. Der passive Hämagglutinationstest (PHA) ist eine zuverlässige Methode für die Feststellung von Antigenen gegen *Herpesvirus hominis*. PHA-Testergebnisse mit denen des CF-Tests verglichen ergaben, dass die Antigene, die zur PHA-Aktivität führen, sich von denen der CF-Antigene und des infektiven Virus unterscheiden lassen.

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Daily Variations in Pigeon Cropsac Responses to Prolactin

Daily variations in responses to prolactin in several vertebrates have been described by our laboratory. For example, daily injections at one time of the day for 6 days resulted in increases (75–500%) in the lipid stores of fish[1], frogs[2], lizards[2], and birds[3], whereas injections at another time of day caused losses (20–60%) in the total body fat. Also, daily variations in responses to prolactin have been described for the growth of fish[1] and lizards[2], locomotor activity of birds[3], and the inhibition of amphibian metamorphosis[4]. Inasmuch as the pigeon cropsac response is the basis of prolactin bioassays, it seemed of interest to learn whether there is a daily variation in the sensitivity of the cropsac.

There are 2 fundamental techniques for assaying prolactin by the cropsac. The systemic (intramuscular) method[5] was tested during June, 1969, using 7–8-week-old white king pigeons. The intradermal method[6] was tested during December, 1969, using 4–5-month-old tumbler pigeons. For each test the birds of mixed sexes were caged in indoor metal coops; the temperature was maintained at 25 ± 2 °C, and a 12-h photoperiod (07.30 to 19.30 h) (100–200 lux at cage level) was supplied. The

birds were kept undisturbed between injections. Water and a commercial pigeon feed were available continuously. The pigeons were allowed to adjust for 1 week before the tests were begun.

For the systemic assay, 100 pigeons were divided equally into 4 groups, 3 that received prolactin and 1 that received saline carrier. Total 4-day doses of 0, 200, 400, or 600 µg of ovine prolactin (1 mg = 28 IU) were injected i.m. Each of the 4 dose-level groups was divided into 5 subgroups that received daily injections at either 0, 3, 6, 9, or 12 h after the onset of the daily photoperiods. On the 5th day of the test (June 30, 1969), 24 h after each group's last injection, the cropsacs were removed, cleaned of fat deposits, and weighed.

For statistical purposes the experimental setup was considered to be a 4 × 5 (4 levels, 0, 200, 400, and 600 µg × 5 times, 0, 3, 6, 9, and 12 h after the onset of the photoperiod) factorial arrangement of treatments in a completely randomized design with 5 pigeons per treatment combination. The random errors were assumed to be distributed normally and independently about a mean of zero with a common variance[7]. Comparisons were made among dose levels (linear, quadratic, and cubic) and among times of day (linear, quadratic, cubic, and quartic) by means of a set of orthogonal polynomials.

As expected, a highly significant difference ($p < 0.01$) was observed between any 2 dose levels (Table I). The cropsac weights increased linearly ($p < 0.01$) as the dose levels increased. Among times of day, a highly significant ($p < 0.01$) linear ($p < 0.01$) and quadratic ($p < 0.05$) difference was observed. In general, the later in the photoperiod the prolactin was injected the greater were the responses (Figure 1). There was a decline, however, from the 9th h to the 12th h. The effects of time of day and dose level were independent of one another, i.e., the level × time interaction was not significant.

For the intradermal assay, 27 pigeons were randomly divided into 3 equal groups. Injections were made daily for 4 days at either 0, 6, or 12 h after the onset of the 12-h photoperiod (08.00–20.00 h). On the right side of the cropsac each pigeon received a 0.1 ml injection of 12.5 µg of ovine prolactin in 85% saline, while on the left side each pigeon received a 0.1 ml 85% saline (control) injection. The total dose of prolactin for 4 days was 50 µg (1 mg = 28 IU). One pigeon in the 0-h group died. The cropsacs were removed during the 5th day (December 27, 1969), 24 h after each group's last injection. Using recently refined techniques[8], 4-cm diameter mucosal scrapings were taken from each side of the cropsac, dried in a vacuum desiccator, and weighed. The intradermal experiment was a split plot 2 × 3 factorial arrangement of treatments in a completely randomized design where times of the day (0, 6, and 12 h after the onset of the photoperiod) are the whole plots with 8 pigeons at 0 h, 9 pigeons at 6 h, and 9 pigeons at 12 h. Dose levels (0 and 50 µg of prolactin) were the split plots. Comparisons were made among the times of day (linear and quadratic) and between dose levels (linear) by means of a set of orthogonal polynomials (Table II).

A highly significant difference ($p < 0.01$) was found between the responses to saline injections as compared to the prolactin injections. Among times, a highly significant linear difference ($p < 0.01$) was found for both the saline and the prolactin injections. Greater responses occurred when prolactin was injected later in the photoperiod (Figure 2). The increase during the day

Table I. Least-squares analysis of variance in cropsac responses to i.m. injections of prolactin (see Figure 1)

Source of variation	Degrees of freedom	Mean of squares
Level	3	12.075[b]
Linear	1	36.051[b]
Quadratic	1	0.019
Cubic	1	0.155
Time	4	6.851[b]
Linear	1	23.763[b]
Quadratic	1	2.250[a]
Cubic	1	0.157
Quartic	1	1.236
Level × time	12	1.481
Error	80	0.784

[a] $p < 0.05$. [b] $p < 0.01$.

Table II. Split-plot, least-squares analysis of variance in cropsac responses to intradermal injections of prolactin (see Figure 2)

Source of variation	Degrees of freedom	Mean of squares
Time	2	862.12[b]
Linear	1	1566.56[b]
Quadratic	1	157.68
Pigeons/time (error)[a]	23	114.03
Level	1	3679.64[b]
Level × time	2	208.70[b]
(error)[b]	23	31.60

[a] $p < 0.05$. [b] $p < 0.01$.

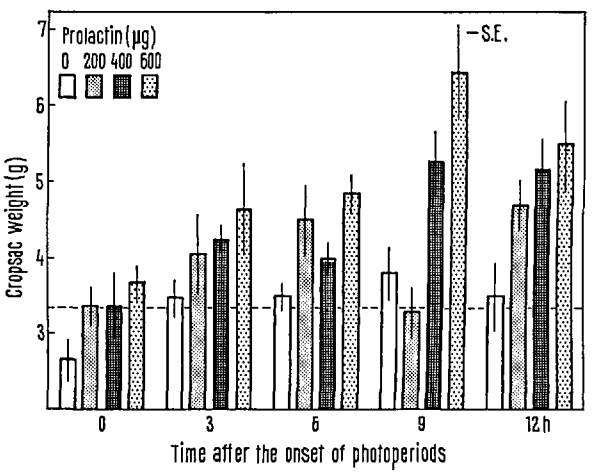

Fig. 1. Daily variations in cropsac responses to i.m. injections of prolactin. 4 dose levels of prolactin (0, 200, 400, or 600 mg) were given in 4 equal daily injections at one of 5 different times during the day (0, 3, 6, 9 or 12 h) after the onset of the daily photoperiods (07.30–19.30 h). The broken line represents the mean level (3.35 g) of the combined controls.

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of the saline injected sides was less than the increase of the prolactin injected sides, i.e., the level × time interaction was significant ($p < 0.01$).

The basis of the daily cropsac variation remains to be explained. However, thyroxin, prednisone, and growth hormone enhance the responses of hypophysectomized young pigeons to prolactin[9]. Daily rhythms in hormone levels have been found in many vertebrates, e.g., TSH in rats[10,11]; corticosterone in mice[12], rats[12], humans[12], and sparrows[13]; prolactin in rats[14], hamsters[15], and sparrows[16]. The rise and fall during the day of hormones auxiliary to a cropsac response might account for a sensitivity rhythm. As suggested for white-throated sparrows[16], sensitivity rhythms in pigeons may be involved in the temporal regulation of prolactin-dependent phenomena. Also, the existence of a marked daily variation in the response of cropsacs requires investigators to consider the time of day when cropsac assays are made[17].

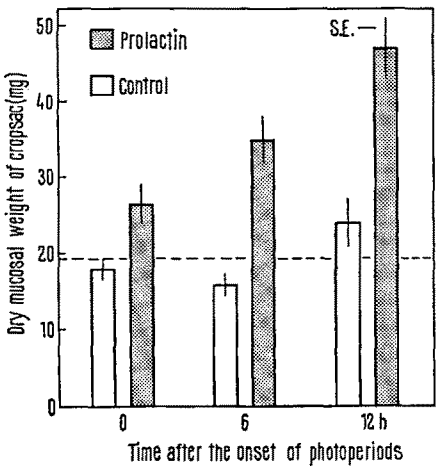

Fig. 2. Daily variations in responses of the cropsac mucosa to intradermal injections of prolactin. Prolactin (50 mg total) and saline were given in 4 equal daily injections at one of 3 different times during the day (0, 6 or 12 h after the onset of the daily photoperiod, 07.30–19.30 h). The broken line represents the mean level (19.2 mg) of the combined controls.

Zusammenfassung. Es wird festgestellt, dass die Ansprechbarkeit des Taubenkropfsackes auf Prolactin in Abhängigkeit vom hormonalen Zustand des Versuchstieres von Tag zu Tag verschieden ist.

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Growth Hormone Release After Hepatectomy[1]

Contradictory reports on the participation of the pituitary gland in liver regeneration have appeared in the literature for some time[2–4]; but recent work has thrown some light on the role played by this gland in this process[5,6].

With the aim of obtaining more information on this subject, and with a different approach, we have, as a first step, looked for changes in the growth hormone-producing cells of the pituitary gland of hepatectomized mice and we have observed morphological changes indicating a massive release of the hormone[7]. These changes reached a maximum at midnight of the second day after hepatectomy, some hours before the appearance of the first peak of DNA synthesis in the regenerating liver (ECHAVE LLANOS et al. unpublished results).

In a second step of which the results are reported here, we have assayed the plasma of hepatectomized mice, taken at midnight of the second day after hepatectomy, the time of maximal release changes in the growth hormone-producing cells, on the DNA synthesis index of the liver of intact adult mice. Its effect was compared with the action of saline, plasma from intact mice killed at midnight and pure bovine growth hormone.

Seventy-two 90-day-old C3H-S inbred male mice were used for the experiment. They were standardized for periodicity analysis[8]. 20 of them, hepatectomized[9] at 16.00 h[7], were killed by decapitation at midnight of the second day of regeneration, together with 20 intact mice. The blood of each group was collected as a pool on 1 ml of 3% sodium citrate and then centrifuged at $3000 \times g$ and 0 °C. The plasma obtained was stored for 16 h at 0 °C until injection.

Four groups of 8 intact mice were used as receivers. They were injected i.p. at 16.30 h for 3 consecutive days, with 0.01 ml/g body weight of the following solutions: 1. Buffered saline (pH: 7.2) with 3% sodium citrate (9:1). 2. Plasma from intact mice killed at midnight. 3. Plasma from hepatectomized mice killed at midnight of the second day after hepatectomy. 4. Growth hormone (NIH-GH-B13. Bovine) dissolved (1 μg/0.01 ml) in alkaline medium, buffered to pH 7.2 and finally added with 3% sodium citrate (9:1). All the animals were killed after the third injection at 02.00 h of the following day, having received, 1 h previously, an i.p. injection of tritiated thymidine (From New England, Chicago. Specific activity: 2 C/m*M*) in a dose of 1 μC/g body weight. The liver was removed and processed for autoradiography. The DNA synthesis index was determined and expressed as labelled nuclei/10,000 nuclei.

In this experimental design (Figure), the time for the injection of the hormone (16.30 h) was chosen bearing in mind the results of HALBERG[10] who demonstrated