previously injected with estrogen revealed extensive lobule-alveolar development with secretion in the alveoli, whereas controls injected only with estrogen showed an extension of the duct system with no alveolar growth. 3. It is probable that reserpine induced an increased secretion and/or release of prolactin and perhaps of other hormones favorable to mammary growth and lactation, but confirmation of this hypothesis awaits assay of pituitary hormones.

Addendum: Pituitary assays now completed reveal that reserpine induced a substantial increase in prolactin in both normal and estrogen-treated rabbits.

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Effects of Growth Hormone on Lactation and Body Growth of Parturient Rats.* (23591)

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Growth hormone (GH) has been demonstrated to increase milk yields in the parturient cow(1,2), sheep(3) and goat(4). Shaw *et al.*(5) reported that when GH was injected into cows on a limited ration, milk production fell for the first 3 days but this was followed by a return to normal levels of milk together with a striking increase in milk fat. This suggests that GH injections into lactating ruminants may preferentially divert nutrients to the mammary glands rather than be used for growth or maintenance purposes. Significant effects of GH on body weight of ruminants have not been reported.

It was of interest to determine whether injections of GH into parturient rats would (a) elicit increases in milk production similar to those observed in ruminants and (b) increase body weight. The ability of GH to increase body weight in mature female rats which have reached growth stasis constitutes an established assay method for this hormone(6).

Methods. Mature female albino rats (Carworth) of uniform age and weight and which had reached growth stasis were bred, and on the day of parturition their litters were reduced to 6 young per dam. On the 5th day after parturition, 20 rats were divided into 2 groups of 10 each. Ten were injected subcutaneously with 1 mg GH† daily in .1 cc distilled water, and the other 10 rats (controls) were injected only with the same volume of distilled water. This treatment was continued until the 17th day after parturition. The rats were maintained in an air-conditioned, temperature controlled room, and both dams and litters were weighed

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† A gift of growth hormone was kindly supplied by The Endocrinology Study Section, N.I.H.

TABLE I. Effects of GH on Average Body Weight of Suckled Rats and Litters.

Treatment	Avg body wt (g) on following postpartum days 1	3	5	7	9	11	13	15	17	Avg wt gain (g), 5th-17th day
Controls										
Dam	268 ± 5.4†	267	264	264	266	268	267	265	264	0 ± 3.5†
Litter of 6	38 ± 1.3	51	69	87	107	129	150	170	186	117.4 ± 5.8
GH*										
Dam	265 ± 5.4	262	265	271	273	280	283	285	288	23.0 ± 3.1
Litter of 6	37 ± .3	49	65	82	105	125	147	170	191	125.5 ± 4.0

* GH started on 5th postpartum day. † Stand. error of mean.

every 2 days.

Results. The mother rats and litters of both groups initially weighed about the same for the first 5 days after parturition (Table I). Injections of GH beginning on the 5th postpartum day induced a steady increase in body weight over the controls. At the end of the 17th day the GH-treated rats had gained an average of 23.0 ± 3.13 g each, or about 2 g per day, while the controls showed no significant change in body weight. Each of the GH-injected rats gained from 10 to 36 g during the experimental period, whereas 5 controls either lost or failed to gain in body weight. The litters of both groups grew at about the same rate during the experimental period, indicating that GH did not enhance milk production.

Discussion. These results suggest that a dose of GH sufficient to induce a significant increase in body weight of suckled mother rats which had reached growth stasis, does not increase milk yield as judged by growth of their litters. Apparently GH is not galactopoietic in the rat in contrast to its action in the cow, sheep and goat. Work in our laboratory has recently demonstrated that injections of prolactin, ACTH or cortisone into parturient rats can increase growth rate of litters(7.8). Furthermore, these hormones were able to partially prevent involution of the mammary parenchyma of parturient rats after their young were removed, whereas GH had no apparent effect on mammary involution.

Several workers have demonstrated that lactation can be initiated in hypophysectomized guinea pigs with a combination of prolactin and ACTH or adrenal cortical hormones(9.10). Lyons (private communication) recently stated that only prolactin and ACTH were essential to initiate lactation in hypophysectomized rats with developed mammary glands. Thus, despite the demonstrated galactopoietic activity of GH in the cow, sheep and goat, it does not appear to be galactopoietic or an essential factor for either the initiation or maintenance of lactation in the rat.

Summary. Twenty mature female albino rats which had reached growth stasis were bred, and on the day of parturition their litters were reduced to 6 young each. On the 5th postpartum day 10 rats were injected with 1 mg GH daily for 12 days and the other 10 rats served as controls. The GH-injected rats gained an average of 2 g each daily during the experimental period whereas the controls showed no increase in body weight. The growth rate of the litters was the same in both groups, indicating that GH did not increase milk production. This contrasts with the demonstrated ability of GH to increase milk production in cattle, sheep and goats.

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Biochemical Changes in HeLa Cells Associated with Infection by Type 2 Adenovirus. (23592)

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Infection of tissue culture cells with poliomyelitis virus has been shown to result in a rapid alteration of cellular metabolism, with temporary increased production of lactic acid and suppression of uptake of radioactive glycine(1). In their overall effects on HeLa cells, adenoviruses present a marked contrast to the poliovirus system previously studied; whereas poliovirus appears to suppress cellular metabolism with overall inhibition of acid production(2), adenoviruses stimulate acid production by infected HeLa cells(3,4) and do not interfere with the incorporation of vital stains for long periods after complete cytopathic effects(5). Consequently, experiments were conducted to determine some of the biochemical changes accompanying adenovirus infection of HeLa cells. A high multiplicity of virus to cells was employed in order to obtain as nearly synchronous infection as possible. Experiments were designed to survey a broad area of biochemical activity, with more detailed observations to follow upon any positive observations. This report will deal with the effect of the time course of the infection on the production of lactic acid by HeLa cells, and on the rate of their uptake of P^{32} and glycine-2-C^{14}, as well as with the distribution of these isotopes among several biochemical fractions after 5 hours of infection.

Materials and methods. HeLa cell cultures. HeLa cells† were grown in 2 oz. or 32 oz. prescription bottles, in a medium consisting of 10% human serum in Eagle's basal medium (BME)(6). In the majority of experiments, all cultures were prepared from a single pool of cells, the tissues were re-fed at 2 or 3 days, and the cultures used after an additional 2 days; only cultures with confluent cell sheets were used. Immediately before virus inoculation, the growth medium was removed and the cultures were rinsed with BME or 3:1 Hanks-Simms solution(7, 8). The 2 oz. bottle cultures were washed 3 times with 5 ml, and the 32 oz. cultures 2 or 3 times with 20 ml. Maintenance medium was 5% chicken serum in Eagle's basal medium, in a volume of 5 ml for the 2 oz. bottles and 40 or 50 ml for the 32 oz. bottles. All media contained penicillin, 250 u/ml, and streptomycin, 250 μg/ml. *Virus.* The Ad. 6 strain of adenovirus type 2, was used throughout. Virus pools were prepared in HeLa or KB cell(9) cultures: 4 pools were used, each of which had an infectivity titer for HeLa cells of $10^{8.5}$ $TCID_{50}$ per ml. The 2 oz. bottle cultures received 0.2 ml, and the large bottle cultures 1.6 ml of the undiluted virus suspension. This dose of virus produced complete cytopathogenic effects with beginning loss of cells into the culture medium within 18 to 24 hours after inoculation of the HeLa cell cultures. Control cultures were inoculated with an equal volume of medium from uninfected cultures of the same type as were used for production of the virus. Both virus and control inocula were clarified by centrifugation at 2,000 rpm for 10 minutes. *Radioactivity.* Radioactive inorganic phosphate was received from the Atomic Energy Commission, Oak Ridge, Tenn. Glycine-2-

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† Seed cultures were obtained from Microbiological Associates, Bethesda, Md.