Direct evidence that synthesis of the surface antigens is under control of the virus genome and the role of these antigens in tumor immunity or in the neoplastic process itself is lacking, but these problems are under investigation.

Summary. Immunization of rabbits with cells transformed by papovavirus SV40 resulted in the production of cytotoxic antibodies specific for the homologous cells. Following absorption with normal hamster embryo fibroblasts, these sera failed to react with normal hamster cells, with BHK21 hamster cells, or with hamster cells transformed by adenovirus 7. Absorption with the homologous SV40 transformed cells removed all activity. The reaction observed was dependent on complement. The results suggest the presence of new specific antigens at the surface of cells transformed by SV40 and support the previous localization of surface antigens by the indirect immunofluorescent method.

ADDENDUM. Since this work was completed, we have observed that absorption of the rabbit antisera with sheep erythrocytes decreases, but does not abolish, the cytotoxic reaction against the H-50 cells.

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Induction of Implantation in the Hypophysectomized Rat with Gonadotrophins.* (30562)

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Implantation of the rat blastocyst takes place on Day 6 of pregnancy. Experimental manipulation of the hormonal environment can cause delayed implantation as previous studies from this and other laboratories have shown(1). Implantation is delayed in the pregnant progesterone-treated ovariectomized (2,3) or hypophysectomized(3) rat. The anterior pituitary gland of the rat, when autografted to the kidney capsule, secretes abundant luteotrophin (Prolactin) with relatively little or no gonadotrophin release(4,5), and if the autograft is performed early in pregnancy, nidation is delayed(6). Administration of a single, small dose of estrogen initiates implantation and subsequent embryonic development in each of these experimental situations. It has been assumed that, since estrogen initiates implantation during the pe-

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riod of delay in the experimental rat, estrogen is responsible for embryo-implantation in the intact pregnant rat.

The availability of gonadotrophic substances of increasing purity permits an examination of the gonadotrophin requirements to produce sufficient estrogen to initiate implantation. This investigation utilizes the pregnant progesterone-treated hypophysectomized rat to compare the effect of rat pituitary homogenates and gonadotrophic hormones of relative purity on induction of implantation.

Materials and methods. Virgin female albino rats (180-200 g) obtained from the Holtzman Co. were maintained on Rockland Rat Diet and tap water *ad libitum*. Animal room temperatures were maintained at 78°F with a 14 hour light-10 hour dark schedule. Females were caged with males in the evening and the vaginal smear examined for the presence of sperm the following morning. The day on which sperm were found in the vagina was designated as Day 1 of pregnancy.

On Day 2 of pregnancy, hypophysectomies were performed by the parapharyngeal approach. Following hypophysectomy, Cerelose (Corn Products Co.) (dextrose sugar) was also offered *ad libitum* and 4 mg progesterone and 400 μg hydrocortisone in corn oil were injected subcutaneously daily. Under this regimen the blastocysts enter and remain in the uterus in a viable but dormant condition.

NIH preparations of ovine FSH, ovine and bovine LH, prolactin and commercial preparations of HCG and PMS were injected into the tail vein of hypophysectomized pregnant rats. Pituitary homogenates from immature and adult rats of both sexes were administered subcutaneously or intravenously *via* the tail vein. Single injections were administered on Day 5 of pregnancy; multiple injections of NIH-LH were given on both Day 5 and 6 as described in the *Results*. The glands used were collected from cycling female rats between 10:30 and 11:00 A.M. on Day 2 of diestrus or proestrus, from 25- to 30-day-old male and female rats, year-old male rats, and male rats that had been castrated for 4 months. They were weighed and frozen. Immediately prior to injection, the pituitary glands were homogenized and diluted to the appropriate equivalent per .25 ml volume of saline.

The effect of gonadotrophic materials on implantation was assessed 5 days later on Day 10 of pregnancy. The presence or absence of implantation sites, size of sites as measured by antimesometrial-mesometrial width, weight of the 2 ovaries and completeness of hypophysectomy were observed. Rats that exhibited no implantation sites either had their uteri removed and flushed to determine the presence and number of blastocysts or they were given 1.0 I.U. HCG or 0.5 μg estrone to induce implantation.

To establish that the gonadotrophins produce their effects through the ovaries, a group of normal pregnant rats was ovariectomized on Day 3, maintained on 4 mg progesterone and given 1.0 I.U. or 10 I.U. HCG on Day 5 and autopsied 5 days later. In a second group of 14 rats hypophysectomized on Day 2, ovaries were removed at intervals following HCG injection on Day 5 to determine the length of time the ovaries were required for implantation.

Results. At autopsy on Day 10 unimplanted blastocysts were flushed from hypophysectomized rats maintained on progesterone. Intravenous administration of purified pituitary gonadotrophins did not induce implantation. (The effects of the larger doses of gonadotrophin are shown in Table I.) FSH in doses of from 25 to 500 μg had no effect and only 2 of 4 rats responded at 1000 μg. LH in doses of from .5 to 250 μg did not cause implantation except in one rat which had one implantation site and 6 unimplanted blastocysts. One rat given the 400 μg LH in 4 injections also had sites.

Gonadotrophins of placental origin were, on the other hand, highly effective (Table I). Implantation was induced consistently by 0.5 I.U. HCG or 2.0 I.U. PMS in hypophysectomized rats. The injection of 1.0 I.U. of HCG 24 hours prior to removal of the ovaries also caused nidation. HCG had no effect if the ovaries were removed earlier than 20 hours after HCG. Injection of 1 or 10 I.U. HCG into rats ovariectomized on Day 3, with

intact pituitaries, failed to induce implantation.

TABLE I. Effect of Gonadotropins on Implantation.

No. of rats	Treatment	No. of rats with sites	No. of rats without sites	Avg No. of sites
6	Saline	0	6	
3	1 mg Prolactin[1]	0	3	
3	5 " "	0	3	
4	5 μg FSH[2]	0	4	
4	1000 " "	2	2	11
3	25 μg LH[3]	0	3	
2	100 " "	0	2	
2	250 " "	1‡	1	1
3	100 " " [4]	0	3	
4	200 " "	0	4	
2	50 " " [4] × 2*	0	2	
2	50 " " × 4†	0	2	
2	100 " " × 2*	0	2	
2	100 " " × 4†	1	1	7
8	.01 I.U. HCG[5]	0	8	
7	.05 " "	7	0	9
4	.1 " "	4	0	12
3	.5 " "	3	0	10
5	1.0 " "	5	0	10
3	10.0 " "	3	0	11
3	.25 I.U. PMS[6]	0	3	
3	1.0 " "	0	3	
3	2.0 " "	3	0	10
3	4.0 " "	3	0	11
3	16.0 " "	3	0	9
3	64.0 " "	3	0	7

[1] NIH-P-S4 [2] NIH-FSH-S2 [3] NIH-LH-S7 [4] NIH-LH-B2 [5] International Hormones, Inc. [6] Gonadogen, Upjohn Co.

* Two injections at 2 and 4 P.M. day 5, total of 100 or 200 μg.

† Four injections at 2 and 4 P.M. day 5 and 6, total of 200 or 400 μg.

‡ One rat had both implantation sites and unimplanted blastocysts.

Large amounts of LH and FSH given concurrently did not consistently cause implantation (Table II); smaller amounts had no effect. Addition of .01 I.U. HCG to 500 μg FSH, levels which individually had no effect, induced implantation, but HCG added to LH induced implantation in only one animal.

The effects of pituitary homogenates are summarized in Table III. The source of the pituitaries appears to affect the response but marked differences were not demonstrated. One half of an immature or adult male pituitary gland induced implantation. Glands from proestrous rats were more potent than those from diestrous ones at the doses injected based either on concentration or total activity per gland.

Discussion. It is thought that gonadotrophins stimulate the ovary to secrete estrogen which in turn acts on the uterus and/or blastocyst to cause implantation. The effect of the exogenous gonadotrophin was shown in this study to be dependent upon the presence of the ovary. An extremely small amount of estrogen is required to induce implantation in pregnant rats in which implantation has been delayed. The minimal subcutaneous dose of estrone to cause nidation in the ovariectomized progestrone treated pregnant rat is between 0.3 and 1 μg(7). A single juxtauterine dose of only 0.0025 μg estradiol will induce implantation in the treated horn in the pregnant, lactating rat(8). This suggests that implantation is a very sensitive indicator of the ability of gonadotrophic sub-

TABLE II. Effect of Combinations of Gonadotropins on Implantation in Hypophysectomized Rats.

No. of rats	Treatment	No. of rats with sites	No. of rats without sites	Avg No. of sites
2	100 μg LH* + 100 μg FSH†	0	2	
2	100 " " + 200 " "	0	2	
4	100 " " + 500 " "	2‡	2	8
4	200 " " + 500 " "	3	1	9
3	100 μg LH + .01 I.U. HCG	0	3	
3	200 " " + .01 " "	1	2	13
3	100 μg FSH + .01 I.U. HCG	0	3	
4	200 " " + .01 " "	2§	2	8
4	500 " " + .01 " "	4	0	11

* NIH-LH-B2.

† NIH-FSH-S2.

‡ One rat had both implantation sites and unimplanted blastocysts.

§ One rat had sites implanted 1 day later than expected.

TABLE III. Effect of Pituitary Homogenates on Implantation in Hypophysectomized Rats.

No. of rats	Treatment	No. of rats with sites	No. of rats without sites	Avg No. of sites
3	1/2 immature ♂ pit. S.C.	3	0	10
3	1 " " " I.V.	2*	1	4
3	1 " " " S.C.	3	0	10
3	1 " ♀ " S.C.	1	2	9
3	1/32 adult ♂ pit. I.V.	0	3	
3	1/8 " " " I.V.	0	3	
4	1/2 " " " I.V.	1	3	13
4	1/2 " " " S.C.	2	2	9
4	1 " " " S.C.	4	0	9
4	1/2 castrate ♂ pit. S.C.	4	0	11
3	1/8 proestrous ♀ pit. I.V.	0	3	
2	1/8 " " " S.C.	1	1	10
3	1/2 " " " I.V.	1	2	11
4	1/2 " " " S.C.	2†	2	7
3	1/8 diestrous ♀ pit. I.V.	0	3	
2	1/2 " " " I.V.	0	2	
3	1/2 " " " S.C.	0	3	

* One rat had both implantation sites and unimplanted blastocysts.
† One rat had sites implanted 1 day later than expected.

stances to stimulate estrogen secretion.

These data reveal no correlation between capacity to induce implantation and gonadotrophic activity as measured in other ways, *i.e.*, ovarian ascorbic acid depletion and induction of ovulation. The minimum effective doses which induce implantation consistently were 0.05 I.U. HCG, or 2.0 I.U. PMS. A minimum dose of NIH-LH, that could be attributed solely to LH activity rather than contamination, was not found. Parlow(9) found that doses of 0.4 I.U. HCG, 0.2 mg LH (Armour Standard) and 2.0 I.U. PMS produced comparable effects in the OAAD assay. In our laboratory, induction of ovulation in the immature rat primed with 15 I.U. PMS was achieved with either 0.5-1 I.U. HCG or 2-3 μg NIH-LH(10 and unpublished data). Less HCG was required to induce implantation than to deplete ovarian ascorbic acid or cause ovulation. NIH-LH, on the other hand, did not have the capacity to induce implantation presumably because of its inability to stimulate sufficient estrogen secretion.

If the ability to induce implantation is dependent on both FSH and LH and if a synergistic relationship exists between them, then the amount of these hormones expected to induce implantation should be significantly less than the amount actually shown to be required in these experiments. However, injections of doses of HCG and FSH, which individually had no effect, did, when combined, cause implantation.

Injection of pituitary homogenates from either male or female immature or adult rats caused implantation of blastocysts in hypophysectomized rats. Induction of implantation is difficult to explain, however, when one compares the amount of NIH-LH and FSH injected to the amount of FSH and LH reported to be present in the pituitaries of the donor rats. Several investigators have reported FSH and LH levels in rat pituitaries. Parlow(11), using the Steelman-Pohley Assay found in the adult male and adult male castrate values of 169 and 161 μg equivalents of NIH-FSH-S1 per mg dry weight of anterior pituitary gland. These pituitaries were also found to have an LH content (OAAD) of 2.8 and 22.1 μg equivalent of NIH-LH-S1 per mg dry weight. Schwartz and Bartosik (12) using the OAAD method for LH found the pituitaries to have 7.8 μg per gland in proestrous and 5.3 μg LH per gland in the diestrous rat. Ramirez and McCann(13) reported about 40 μg LH per adult male gland and 7.0 μg in the immature male pituitary gland. Comparable LH activity has been found by Anderson in our laboratory (personal communication). Even though the data

from different laboratories vary somewhat, rough approximations indicate that in our experiments 10 times as much NIH-LH-S1 equivalent was injected as has been demonstrated to be present in rat pituitary by the OAAD method.

Rat pituitary homogenates and low levels of HCG and PMS were able to induce implantation despite the fact that the rats had been hypophysectomized from Day 2 of pregnancy until gonadotrophic injections on Day 5. It was also observed that the ovaries responded to a single injection of 1.0 I.U. of HCG 15-17 days after hypophysectomy, as shown by the presence of implantation sites 5 days after HCG injection. It may be postulated that either the corpora lutea or small follicles or interstitial tissue are responsible for the estrogen secretion causing implantation. Preliminary work in this laboratory suggests that corpora lutea transplanted to the kidney capsule in the ovariectomized progesterone treated pregnant rat are capable of secreting sufficient estrogen to induce implantation. This concept was also supported by Greep(14) who observed estrogen secretion in hypophysectomized rats after single injections of HCG, but not when given pituitary preparations. He concluded that the corpora lutea were necessary for HCG stimulation of estrogen. Parlow(15) found that immature rats made pseudopregnant with PMS and HCG, then hypophysectomized and treated with NIH-LH or HCG, secreted estrogen as determined by vaginal smears. He concluded the site of action of HCG was the corpus luteum since rats not made pseudopregnant, and therefore without corpora, did not respond to HCG after hypophysectomy.

Recently, in an effort to demonstrate estrogen secretion from ovaries secreting progesterone, it was shown that 50 μg NIH-LH-S1 per day for 4 days induced implantation in the hypophysectomized pregnant rat bearing a pituitary autograft(16). Barnes has observed induction of implantation in rats, in which nidation was delayed with Provera (17), after administration of 3 daily doses of 5 I.U. HCG (personal communication).

The reason for the low activity of the purified pituitary preparations as contrasted to the high activity of the placental gonadotrophins and the efficacy of the pituitary homogenates may be that the species source of gonadotrophin is of such importance as to make even large doses of ovine or bovine pituitary preparations ineffective. A second factor to consider is the rate of disappearance of these hormones from the blood or "biological half-life." Parlow(18) calculated values of 26 hours for PMS; 4.9 hours for HCG and 15 and 17 minutes for sheep LH and rat LH. Four injections over a 2-day period of NIH-LH were not effective, however, in inducing implantation.

Summary. Pituitary homogenates and very low levels of placental gonadotrophins induced implantation in the hypophysectomized pregnant rat maintained on progesterone. However, implantation was not induced by purified pituitary gonadotrophins at doses consonant with their activity as measured in other ways.

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Effect of Diphenylhydantoin on Proline and Hydroxyproline Excretion in the Rat. (30563)

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Increased total dermal collagen deposition was induced in the young rat by diphenylhydantoin (DPH) administration in experiments performed by Houck *et al*(1). Increase in less soluble (acid soluble and insoluble) collagen was responsible for the observed increase in total collagen. Simultaneous decrease of more soluble (salt soluble) collagen occurred. DPH thus has the reverse effect from that induced in experimental lathyrism in young rats in which dermal salt soluble collagen was increased; however, total dermal collagen remained constant(2).

Peptide bound hydroxyproline excretion has been shown to reflect the metabolic degradation of collagen, the major site of which is the soluble fraction of collagen(3). Because an increase in bound hydroxyprolinuria accompanied the delayed appearance of increased soluble collagen pool in lathyritic rats(2), it seemed of interest to determine whether the excretion of hydroxyproline (HP) and its precursor proline were altered in DPH treated rats.

Methods. Eight-week-old male Sprague-Dawley (Holtzman) rats weighing 220-260 g were treated with either diphenylhydantoin, 15 mg, or propylene glycol-ethanol vehicle, 0.2 ml, daily by intraperitoneal injection for one week. The rats were weighed daily and fed Purina Lab Chow containing trace amounts of HP and 1.57% proline. Twenty-four hour urine collections were carried out every other day, yielding 4 collections per rat. On the morning of the eighth day, the animals were exsanguinated one-half hour after the usual intraperitoneal injection plus pentobarbital.

Plasma and urinary hydroxyproline levels were determined upon hydrolysed and unhydrolysed samples by the method of Prockop and Udenfriend(4). Proline determinations on similar specimens were made by an adaptation of the methods of Troll and Lindsley (5) and of Messer(6). Briefly, 1 ml of diluted urine, plasma filtrate or hydrolysate, the latter brought to pH 7.0 with potassium hydroxide, was added to 1 ml glacial acetic acid containing glycine 10 μm/ml. Thereafter, 1 ml ninhydrin solution, freshly prepared and containing 20 mg ninhydrin per ml in a mixture of 3 parts glacial acetic acid to 2 parts 6 M phosphoric acid, was added. The covered tubes were then heated in a boiling water bath for 30 minutes. After cooling the tubes in cold water, the color was extracted into toluene, 3 ml. The phases were separated by centrifugation and the supernatant read at 515 μ in a Coleman Junior Spectrophotometer. Standards were similarly treated. Concentrated HCl was added to blanks and standards when values for hydrolysates were determined, since the necessary neutralization step caused mild quenching of color. Proline recoveries averaged 96% in unhydrolysed urine, 92% in hydrolysed urine, and 93% in hydrolysed plasma.

Urine creatinines were determined by the method of Peters(7). The data for urinary values were calculated by analysis of variance. Data on plasma levels were treated by the student's T test.

Results. Growth: DPH therapy retarded growth of rats during the experimental period. The mean weight gain of the treated group was 16 g compared to 38 g in the vehicle-treated group. During the week prior to the experiment, the mean gain of all the