sponse in those animals which were sacrificed was equally severe in both groups. The latter finding is in contrast to previous observations made on animals which received living vaccine by the oral route.

The living vaccine was employed to rule out the possibility that our inactivating procedures had destroyed a labile protective antigen. The animals tolerated the living vaccine well, but even under conditions where the same living, virulent organism was used to immunize as was employed to challenge, evidence of protection was not apparent. Thus, parenterally administered preparations conferred no protection, therefore reproducing under our conditions of laboratory assay the results obtained in field studies.

Summary. Monkeys injected subcutaneously with either a combination of heat-killed or acetone-killed *S. flexneri* 2a vaccines or with vaccines made from living, virulent *S. flexneri* are not rendered resistant to experimental oral challenge with the homologous organism.

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Chorionic Gonadotrophin in the Blood and Urine of Pregnant Rhesus Monkeys (*Macaca mulatta*).* (32088)

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Chorionic gonadotrophin has been demonstrated in the urine of pregnant rhesus monkeys(1,2). These studies indicate, however, that monkey chorionic gonadotrophin (MCG) is excreted for only a limited period of pregnancy. The main object of the present study was to develop a sensitive biological test for MCG in order to detect early pregnancy and to measure daily fluctuations in urinary chorionic gonadotrophin.

Materials and methods. Twenty-four-hour urine samples from feral pregnant monkeys were collected in glass jars packed in ice. The urine was filtered through glass wool and either extracted immediately or frozen if extraction was to be done later.

A modification of the procedure described by Bradbury *et al*(3) and Albert(4) for concentrating the gonadotrophic hormones was used for urine extraction. The 24-hour urine volume was recorded and the pH adjusted to 4.5 with 20% HCl. Five ml of a 20% suspension of acid washed Kaolin were thoroughly mixed with the urine and the mixture centrifuged for 5 minutes. The supernatant was discarded and the precipitate washed by adding 10 ml of distilled water and recentrifuging. The supernatant was again discarded and 5 ml of 0.1 N NaOH added to the precipitate, mixed thoroughly and centrifuged for an additional 5 minutes. The supernatant was saved and the pH adjusted to 7.0 with 1% HCl.

Twenty-one-day-old female rats (Holtzman strain) were used as bioassay animals and

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the increase in uterine weight taken as the end point for hormonal activity. Standards and urine extracts were administered in 5 subcutaneous injections given over a period of 2.5 days. Each treatment group consisted of 3 rats. The animals were killed by cervical dislocation, the uteri carefully dissected and firmly pressed in a fold of filter paper to express the luminal fluid. The uteri were then weighed on a Mettler balance to the nearest mg.

Two doses of the HCG† standard (0.5 and 1.0 IU) were included in each assay and the uterine weight plotted. Various dilutions of the 24 hour urine extract were made. Only those dilutions which induced a uterine weight increase falling within the range of the standard HCG dose response curve were used to determine the total 24 hour output of MCG which was expressed in terms of HCG IU.

The sensitivity of the assay was increased during the periods when MCG in the urine would be expected to be low, by mixing the urinary extract with either human eunuch gonadotrophin (HEG;NIH-HPG-UE)‡ or human postmenopausal gonadotrophin (HMG; NIH-HPG-UPM-1)‡ prior to injection. In these experiments, the HCG standard was run at 2 dose levels (0.125 and 0.25 IU) and was supplemented with 0.1 mg of either HEG or HMG (Fig. 1). When the primary objective was to detect early pregnancy regardless of the amount of MCG, 0.1 mg of HEG or HMG was added to the extract obtained from an 8-hour sample of urine and injected into each assay animal. A 70% or greater increase in uterine weight over that of controls was regarded as a positive indication of pregnancy.

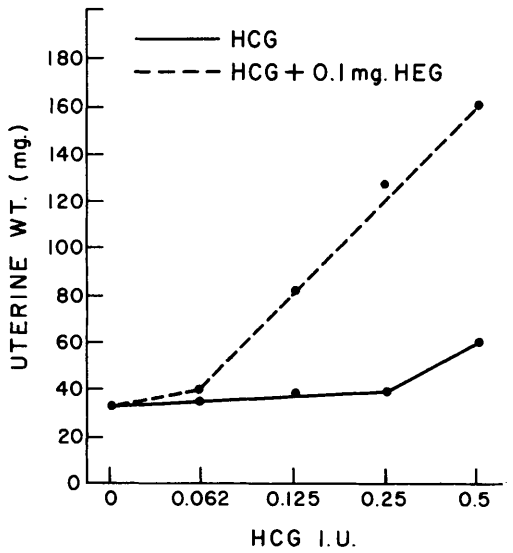

FIG. 1. Dose response curve of HCG with and without HEG augmentation.

† Obtained from International Hormones, Inc.

‡ Obtained from Endocrinology Study Section, NIH.

Serum was also obtained from monkeys at various days after mating. Dilutions of the untreated serum were made with physiological saline and injected subcutaneously in test animals according to the regimen described for injecting urinary extracts. Urine extracts and sera from non-pregnant animals were also tested.

Results. Levels of MCG in urine. Urinary extracts from 3 monkeys (774, 797 and 710) were assayed for chorionic gonadotrophin daily or on alternate days. The 24-hour excretions of MCG of these animals from the 10th to 40th day of pregnancy are presented in Table I. In these animals MCG was first detected on day 12 or 13, and was present in measurable amounts to day 38 or 39 of pregnancy. Periods of highest chorionic gonadotrophin output were from days 23 to 26 in monkey No. 774, days 18 to 28 in No. 797 and from days 18 to 26 in No. 710.

Monkey No. 597 aborted on day 19 of pregnancy. From day 14 to 18 measurable amounts of chorionic gonadotrophin were present in the urine of this monkey but from day 19 no chorionic gonadotrophin could be detected.

The possibility of ovarian uterotrophic factors in the urine extracts was tested by injecting the extracts into a group of ovariectomized immature rats and measuring the uterine response. Extracts were obtained from urine collected during days 20 to 33 of pregnancy, *i.e.*, when the quantity of chorionic gonadotrophin was greatest. In all ovariectomized animals injected with urinary extracts from pregnant monkeys no change in uterine weight was observed.

TABLE I. Urinary Chorionic Gonadotrophin in Pregnant Monkeys.*

Day	No. 774	No. 797	No. 710	No. 597†
10	0	—	0	—
11	0	0	—	—
12	0	0	.3	0
13	.3	.4	—	—
14	.7	.3	1.8	.7
15	.8	—	—	—
16	3.5	.4	12.0	2.2
17	4.8	18.0	—	—
18	7.2	115.2	48.0	7.3
19	7.4	99.8	—	0
20	—	288.8	147.8	—
21	13.9	288.8	205.4	0
22	32.1	211.2	—	—
23	243.8	168.9	67.2	0
24	99.8	172.8	—	—
25	105.6	62.4	—	—
26	48.0	38.4	60.0	0
27	25.9	49.9	—	—
28	28.8	72.0	36.9	—
29	28.8	15.3	—	—
30	23.0	9.1	16.6	0
31	5.5	6.7	—	—
32	3.5	1.8	15.2	—
33	1.5	1.4	7.2	—
34	1.1	.8	—	—
35	.5	.7	.8	—
36	—	.3	—	—
37	.3	.3	.5	—
38	0	0	.4	—
39	0	0	—	—
40	—	0	0	—

* MCG in terms of IU of HCG/24 hours.
† Aborted on day 19.

Levels of MCG in blood. Serum was assayed for chorionic gonadotrophin in 2 monkeys on various days of pregnancy. Since blood was not drawn every day, the exact time of appearance and disappearance of MCG could not be estimated. The results, however, clearly indicate that serum MCG was present on days 20 through 26 of pregnancy (Table II). During this period 0.125 ml of serum was sufficient to induce a statistically significant ($p<.05$) uterine response.

TABLE II. Chorionic Gonadotrophin in the Serum of Pregnant Monkeys.

No. 774		No. 710	
Day	MCG/ml*	Day	MCG/ml
10	.24	10	0
15	.64	15	.42
19	1.50	21	9.60
24	5.12	26	8.40
30	0 †	33	0
35	0	37	0

* In terms of HCG IU.
† Not detectable in 1 ml of serum.

MCG vs *HCG.* To justify the expression of MCG in terms of HCG units, urinary extracts from pregnant monkeys were assayed against the HCG standard. The nonparallelism between the two curves was insignificant ($p>0.05$).

Discussion. Recently Tullner and Hertz (2) have used a semi-quantitative method to determine urinary chorionic gonadotrophin levels in pregnant rhesus monkeys. These authors were not able to detect MCG in urinary extracts earlier than the 15th day of pregnancy. The maximum that they reported at any time was equivalent to 200 IU of HCG per 24 hours. The assay procedure used in the present investigation enabled us to determine pregnancy as early as 12th or 13th day postmating. In these experiments the maximum amount excreted during the peak period ranged between 205 IU to 288 IU/24 hours. In the rhesus monkey as shown by Tullner and Hertz(2) and us, chorionic gonadotrophin is excreted in relatively large amounts only for a limited time during early pregnancy, the same as in the human female. In the latter, however, the hormone is excreted in small quantities throughout the remainder of pregnancy, but this is not true for the monkey. In monkey the time of regression of Langhan's cells, reported to be responsible for the secretion of chorionic gonadotrophin in man(5,6), coincides approximately with the decline in chorionic gonadotrophin in the urine at 38-40 days as reported in the present study. As mentioned above, the augmentation assay detected small amounts of MCG as early as the 12th day of pregnancy. This does not imply necessarily that before this period no chorionic gonadotrophin is secreted by the developing trophoblast. It is quite likely that the trophoblast begins to secrete gonadotrophin earlier, but that the secretion cannot get into the circulation in sufficient amounts until a more intimate association of the foetal villi with maternal circulation has taken place. It has been shown(6) that the differentiation of trophoblastic lacunae filled with maternal blood begins after the 11th day and the process is completed not earlier

than the 15th day of pregnancy.

Summary. A sensitive biologic test for monkey chorionic gonadotrophin (MCG) has been developed based upon the augmentation of MCG either by human eunuch gonadotrophin or human postmenopausal gonadotrophin, using uterine weight of immature rats as the end point. With this method MCG was detected as early as 12 days postmating. The duration of MCG excretion in urine was from day 12 to day 38. The amount excreted reached a maximum between days 18 and 28. The maximum levels were between 205 IU and 288 IU of HCG per 24 hours.

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Effect of Fat and Cholesterol Ingestion Upon Formation and Storage of Lipids and Cholesterol from Acetate-1-C^{14}.* (32089)

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A diet containing large amounts of polyunsaturated fatty acids (PUFA) as a replacement for one with an excess of saturated fatty acids appears to be associated with lowered blood lipid and cholesterol levels(1,2). Several theories have been advanced as possible explanations as to why PUFA ingestion may under certain conditions lower blood serum lipid and cholesterol levels. Bloomfield(3) proposed that the serum cholesterol level was decreased by an altered partition of it between the blood and liver with an increase in cholesterol of the liver. Another theory involved the suggestion that the lowered cholesterol may be the result of a decreased cholesterol synthesis induced by the metabolism of PUFA as a result of an increased efficiency of system involved(1). The report that extrahepatic tissues such as intestinal tissue may synthesize cholesterol(4) suggests that tissues other than the liver may play some role in effecting blood cholesterol levels, although the liver is believed to be the major source of blood cholesterol(5,6).

This investigation was undertaken in an attempt to learn more about the effect of cholesterol ingestion on the effect of PUFA intake upon the formation of total lipids and cholesterol in various tissues of rats. The storage in these tissues of lipid and cholesterol as well as their activities resulting from their formation during an interval after an injection of labeled acetate were determined. Such information should indicate whether there was a difference in the action of saturated and unsaturated fatty acids upon the disposition of cholesterol in the tissues after ingesting a cholesterolemic diet.

Materials and methods. Eight male albino rats weighing about 160 g had been maintained on commercial rat pellets prior to initiation of this experiment. First 4 rats were fed a diet of ground rat pellets‡ enriched with 20% lard, 1% cholesterol and 0.5% sodium taurocholate. The remaining 4 rats were fed a similar diet except 20% lard was replaced by 20% corn oil. All rats were fed 10 days. Be-

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‡ Uncle Johnny's BSD Laboratory Animal Diet, containing not less than 26.0% protein, 5% fat, was prepared by Uncle Johnny Mills, Houston, Texas.