Studies of the Secretion of Monkey Placental Lactogen

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ABSTRACT A radioimmunoassay for monkey placental lactogen (MPL) was developed to study the factors controlling the secretion of MPL. The sensitivity of the assay was 1 ng MPL per ml. Human and monkey growth hormone, and human placental lactogen (HPL) showed minimal cross-reactions with MPL. Maternal MPL concentrations as measured in 40 rhesus monkeys increased progressively throughout pregnancy to a mean of 5000 ng/ml at term while umbilical vein MPL was less than 50 ng/ml. After term delivery maternal MPL concentrations decreased rapidly with a t½ of 20 min.

After fetectomy but with retention of the placenta, MPL concentrations decreased by 25% reaching a plateau over a 6 hr period. Experimental abruption of the secondary placenta within 1 hr produced a 50% decrease in MPL concentration. After ligation of the fetal vessels supplying the secondary placental disc, MPL increased transiently and then decreased to levels significantly below those of the control period.

These studies suggest MPL secretion is not directly controlled by the fetus but is sensitive to changes in placental blood flow. The pregnant rhesus monkey serves as a useful model for investigating factors which may regulate HPL secretion because of the close similarity between MPL and HPL secretion.

INTRODUCTION

Numerous studies of the concentration of human placental lactogen (HPL)[1] have been made in normal and abnormal pregnancies in attempts to define its function in pregnancy. However, the precise biological significance of placental lactogen during pregnancy remains obscure. It is not possible to subject pregnant humans to the variety of experimental manipulations which might be required to explore the many factors which influence HPL synthesis and secretion. Therefore, a radioimmunoassay method has been developed for monkey placental lactogen (MPL) to study various factors which influence its secretion in pregnancy. If the secretion of monkey and human placental lactogens is similar, it may be possible to create experimental situations in monkeys which would lead to a better understanding of the physiological role of placental lactogen in both human and simian pregnancies.

The existence of placental lactogen in monkeys has already been established (1-4). MPL is more closely related immunologically to human growth hormone (HGH) than HPL is (4) and MPL exerts a more potent growth-promoting effect than does HPL. A radioimmunoassay for MPL has been described (5) in which antiserum to human growth hormone and HPL-^{131}I were used to measure MPL in placental extracts but no studies of MPL concentrations in pregnancy have been reported. We developed a homologous radioimmunoassay using guinea pig antiserum to a highly purified preparation of MPL and MPL-^{131}I as a tracer. Using this assay we have studied a variety of factors which influence the secretion of MPL.

METHODS

Antigens. MPL was purified according to the method of Shome and Friesen (6) by use of term rhesus monkey placentas obtained through the cooperation of a number of primate centers. The homogeneity of the MPL preparation has been established by a variety of conventional methods. HGH and HPL were prepared in our laboratory according to methods already described (7). MGH was obtained from rhesus monkey pituitaries. Guinea pigs and rabbits were immunized weekly with 1 mg of each antigen along with complete Freund's adjuvant for a 4 wk period. 1 wk later a booster dose of the antigen was administered intravenously and the antiserum was obtained 10 days later.

Iodination. Iodination was carried out according to the method of Hunter and Greenwood (8). The MPL-^{131}I was

[1] *Abbreviations used in this paper:* HCG, human chorionic gonadotropin; HGH, human growth hormone; HPL, human placental lactogen; MGH, monkey growth hormone; MPL, monkey placental lactogen.

separated from free ^{131}I on a Sephadex G-50 (1 cm × 10 cm) using 0.05 M barbital buffer. In general the efficiency of iodination ranged between 40 and 60%. The specific activity of the MPL-^{131}I was 150–300 μCi/μg. Iodination damage was considerable if the reaction was allowed to proceed more than 5–10 sec after the addition of chloramine T. The fraction containing MPL-^{131}I from the G-50 column was combined with an equal volume of male monkey serum and fractionated on Sephadex G-100 (2 cm × 40 cm) using barbital buffer; 5-ml fractions were collected. The MPL-^{131}I which was damaged and aggregated emerged in the void volume followed by MPL-^{131}I and occasionally a small amount of ^{131}I. Portions of MPL-^{131}I in the second peak were tested with excess antibody to MPL and only those fractions where 80–90% of the MPL-^{131}I was immunoprecipitable were used for the assay.

Radioimmunoassay. A double antibody radioimmunoassay was developed according to the method of Beck, Parker, and Daughaday (9) using guinea pig antiserum to MPL and rabbit anti–guinea pig gamma globulin fraction as the second antibody.

All dilutions were made with 2.5% bovine serum albumin in barbital buffer. 50 μl of samples (standards or serum samples) were added to 0.5 ml of the diluent along with 0.1 ml of MPL-^{131}I and 0.1 ml of the first antibody which was diluted 1/20,000. After incubating the samples for 72 hr at 4°C, 0.1 ml of a 1/200 dilution of normal guinea pig serum and 0.1 ml of the second antibody were added followed by an overnight incubation at 4°C. The samples were counted, centrifuged at 3000 g for 30 min, the supernatants decanted, and the precipitates counted in a Packard Autogamma Counter. When no MPL was present in the standards 60–70% of the MPL-^{131}I added was precipitated in the assays. In the hybrid assays the same procedures were employed except that 0.1 ml of a 1/6000 dilution of a guinea pig antiserum to HPL was used. HPL was measured by radioimmunoassay (9) and HCG was measured by a similar procedure using human luteinizing hormone as standard (potency 4 U/mg NIH LH standard).

Fig. 1 shows that similar amounts of MPL, HPL, and HGH in the hybrid assay do not inhibit the binding of
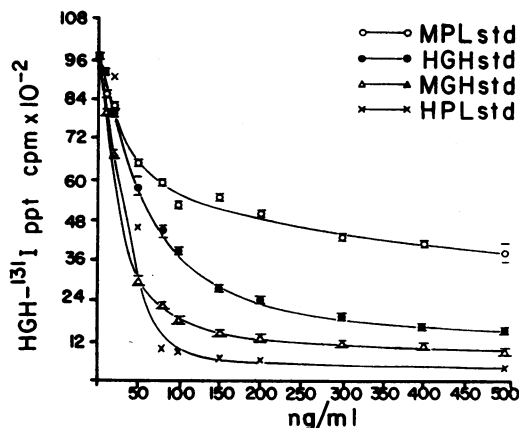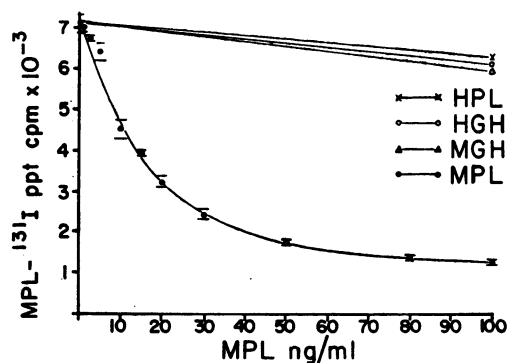
FIGURE 2 The effect of increasing concentrations of unlabeled MPL (●——●), MGH (△——△), HGH (○——○), and HPL (×——×) on the binding of MPL-^{131}I by anti-MPL serum (1:16,000 dilution). The per cent of MPL-^{131}I precipitated is 67% in the absence of any added hormone.

HGH-^{131}I to anti-HPL serum in the same manner. Hence, neither HGH nor HPL can serve as an appropriate standard for measuring MPL concentrations. Moreover, the inhibition of binding of the HGH-^{131}I tracer to anti-HPL antibody by MGH exceeded that of MPL. Therefore, one could not expect to measure low levels of MPL in the presence of MGH using anti-HPL antibody.

Fig. 2 shows a typical standard curve characterizing the homologous radioimmunoassay. Neither HPL, HGH, nor MGH caused significant displacement of MPL-^{131}I binding to its antibody unless the concentration of the three exceeded 100 ng/ml. On the other hand, as little as 10 ng/ml of unlabeled MPL caused significant displacement of MPL-^{131}I binding. Fig. 3 shows that serial dilutions of a monkey placental extract, pregnant monkey serum, and purified MPL inhibit MPL-^{131}I binding equally, whereas, there is little

FIGURE 1 The effect of varying concentrations of unlabeled MPL (○——○), HGH (●——●), MGH (△——△), and HPL (×——×) on the binding of the HGH-^{131}I by anti-HPL serum (1:6000 dilution). The per cent of HGH-^{131}I precipitated is 70% in the absence of added hormone.
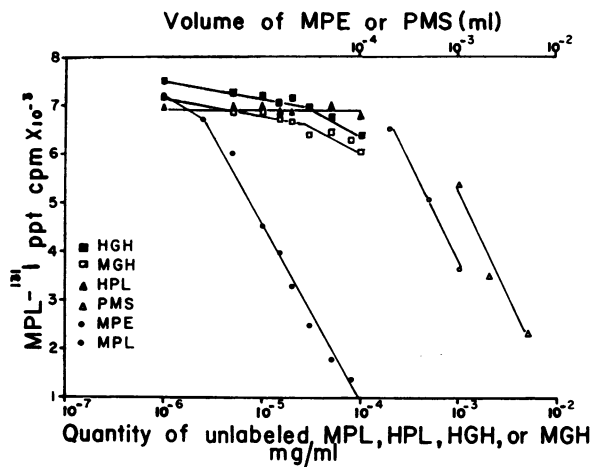
FIGURE 3 The effect of increasing concentrations of unlabeled MPL (●——●), HPL (△——△), HGH (■——■), MGH (□——□), monkey placental extract (MPE) (○——○), and pregnant monkey serum (PMS) (△——△), on the binding of MPL-^{131}I by anti-MPL serum (1:20,000 dilution). The per cent of MPL-^{131}I precipitated is 65% in the absence of any added hormone.
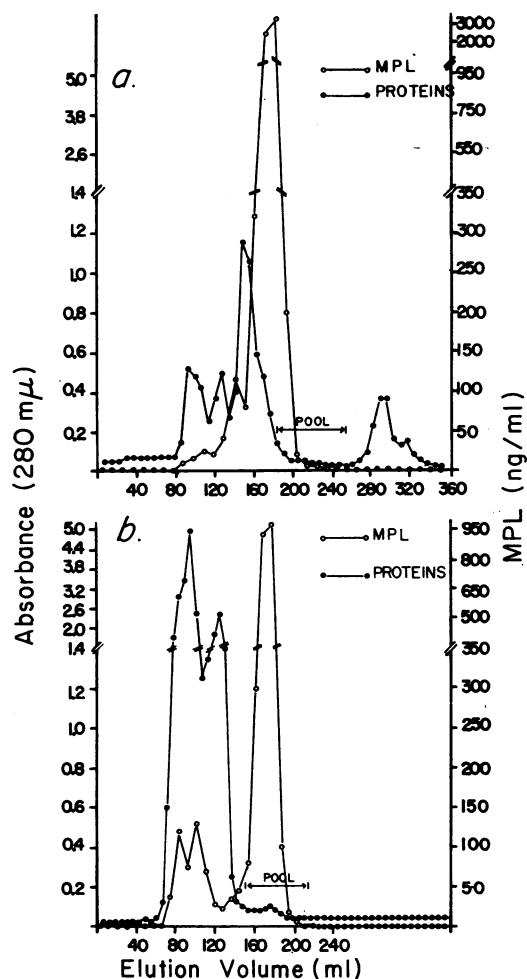
FIGURE 4 Absorbance of proteins (●——●) and distribution of MPL (O——O) after fractionation on a column of Sephadex G-100 (100 × 2 cm) of 2 ml of monkey placental extract (a) and 2 ml of pregnant monkey serum (b) separately. The MPL in the monkey placental extract and in pregnant monkey serum has the same elution volume (140–200 ml). Note the presence of a small but significant peak of cross-reacting material in the monkey serum found at an elution volume 80–120 ml. In each case, a portion of the fractions containing MPL was pooled and lyophilized for examination upon disc-gel electrophoresis.

interference in the radioimmunoassay by the three other antigens, MGH, HGH, and HPL.

To establish the specificity of the assay further we measured the distribution of MPL after gel filtration of a crude monkey placental extract on Sephadex G-100 (Fig. 4 a). The crude monkey placental extract was derived from a much larger extract in which 1177 g of fresh frozen placenta were extracted in 3800 ml of 0.1 M ammonium hydroxide at pH 8.6. The pH was lowered to 5.8 with HCl; the precipitate which formed was removed by centrifugation. To the clear supernatant was added solid ammonium sulfate to make it 50% saturated, with respect to salt. The precipitate was collected by centrifugation, dissolved in 0.1 M ammonium

bicarbonate, and dialyzed against distilled water and lyophilized. An amount of powder equivalent to 0.6 g of frozen placental tissue was dissolved in 2 ml of 0.1 M ammonium bicarbonate and applied to Sephadex G-100. The cross-reacting material had the same elution volume as HPL and purified MPL (140–200 ml). Moreover, the distribution of MPL in fractions after gel filtration on Sephadex G-100 of 2 ml of pregnant monkey serum (Fig. 4 b) was similar to that observed in extracts of monkey placenta. However, in addition to the principal peak of MPL in the elution volume 160–200 ml, a small but significant peak of cross-reacting material was found in the elution volume 80–120 ml. Whether this material represented MPL which was partially adsorbed to serum proteins or an aggregate of MPL is unclear from these studies.

Disc electrophoresis in acrylamide gel was performed according to the method of Davis (10) using Tris buffer (pH 8.2) and tetramethylenediamine to facilitate polymerization of the large pore gel. The sample (50–100 μl) was applied to the column in 0.4 M sucrose and electrophoresis was carried out at 5 ma per tube. Replicate gels were either stained with Amido Schwartz in 6% acetic acid or cut into 2 mm segments which were placed in 1 ml volumes of 0.05 M barbitone buffer (pH 8.6) and frozen and thawed once. The immunoreactive material eluted from each segment after 3 days at 4°C was estimated by radioimmunoassay. The electrophoretic distribution of MPL in a placental extract, pregnant monkey serum extract, and MPL standard were determined (Fig. 5). MPL in the placental extract has an R_f which is approximately similar to the R_f of the MPL standard. The MPL-like material in the serum extract was resolved into two
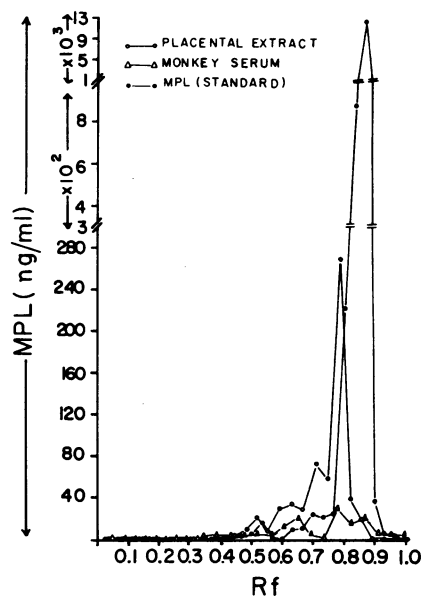

FIGURE 5 Distribution of MPL after acrylamide-gel electrophoresis of monkey placental extract (O——O), pregnant monkey serum (△——△), and purified MPL (●——●). Most of the cross-reacting material in both the placental extract and the serum have an R_f which is approximately similar to that of MPL standard (0.8). Note the two components in the serum extract, one with an R_f of 0.65 and the second with an R_f of 0.8.

components, one with an R_f of 0.65 and the second with an R_f of 0.80. In this acrylamide-gel HPL has an R_f of 0.60 (not shown). It appeared, therefore, that most of the cross-reacting material in both the placental extract and the serum had similar physical and chemical characteristics as the MPL standard.

To determine the content of HPL, HCG, and MPL, fresh frozen human and monkey placental tissue at term were extracted in 0.1 M ammonium bicarbonate at room temperature (20 ml/g). The extract was centrifuged and aliquots of the clear supernatant were analyzed for the three hormones. In addition, 20- to 30-mg fragments of placental tissue were incubated in Krebs-Ringer bicarbonate buffer pH 7.4 with 95% O_2 and 5% CO_2 at 37°C as described (4). At 24 hr, the concentration of the three hormones was determined in the media.

Animals. Pregnant rhesus monkeys were studied under a variety of circumstances at various periods of gestation. The rhesus monkeys were from the breeding colony of the Laboratory of Perinatal Physiology in San Juan, Puerto Rico. All blood samples were obtained without anesthesia except during the course of operative procedures. Samples were obtained from peripheral veins either in the upper or the lower limb.

Operative procedure. Monkeys were operated upon under pentobarbital general anesthesia and sterile conditions. The techniques used in the ligation of the fetal umbilical vessels bridging between the primary and secondary placental discs and supplying the secondary disc with fetal blood circulation have been described earlier (11). Experimental abruption was produced by making a 2.5 cm incision in the uterine wall over the site of attachment of the secondary disc. The gloved right forefinger was inserted between the uterine wall and the secondary disc. Separation of this disc was produced by pressing the finger between the uterine wall and the disc totally interrupting the spiral arterial and draining venous systems. Fetectomies were performed by making a 3.5–4.0 cm incision in the uterus, avoiding the placental discs. The fetus was delivered through this incision, the umbilical cord doubly tied and severed, and the fetus discarded. The uterine incision was carefully repaired leaving the amniotic fluid *in situ.* The animals were returned to their cages while still anesthetized. Depot form penicillin was used 600,000 U and crystalline penicillin 600,000 U was dissolved in the amniotic fluid before repair to combat infection. Without exception after fetectomy, all placentas were retained until surgically delivered at later times as shown in Fig. 9.
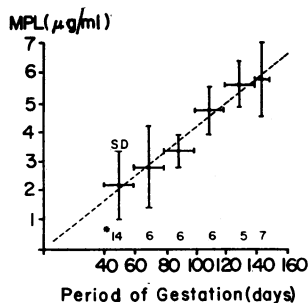

MPL(μg/ml)

FIGURE 6 Serum MPL measured in normal monkeys throughout pregnancy. The mean of MPL concentration and 1 SD for each 20 day period is plotted. The asterisk refers to the number of samples in each 20 day period.
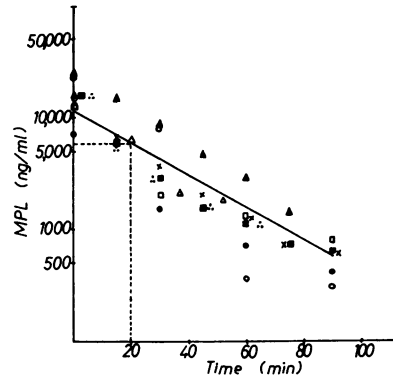

FIGURE 7 41 blood samples were obtained from eight monkeys between 0 and 90 min, after removal of placenta at term. Serum MPL concentrations were determined and plotted on semilogarithmic paper against the time when the blood samples were drawn. The least square regression line of y on x was calculated: $y = 4.05165 + (-0.01439)\ x$, and plotted. The mean half-life of MPL was found to be 20 min.

RESULTS

The maternal serum MPL concentrations in monkeys throughout pregnancy are presented in Fig. 6. At 50 days of gestation, the mean concentration of MPL was 2 μg/ml increasing gradually during pregnancy to a mean of 6 μg/ml at term. The MPL concentration at term ranged from 4 μg/ml to 8 μg/ml. It should be noted that these serum specimens were 2 yr old when MPL measurements were made. The mean MPL concentration in this group at term is somewhat lower than in several recent experiments in which the MPL concentration of fresh serum samples from pregnant monkeys at term were measured.

The maximum concentration of MPL in serum from the umbilical vein was 12 ng/ml in seven different monkey fetuses at term delivery.

MPL concentrations were measured in serum samples taken from the femoral vein before and every 15 min after delivery by caesarian section at term of eight normal pregnant monkeys. The half-time disappearance rate of MPL was calculated by the regression analysis method. The MPL concentration declines with a half-life of approximately 20 min during the first 90 min after removal of the placenta (Fig. 7). Only a few samples were obtained at much later periods (24 and 48 hr) at which time MPL was still detectable, suggesting that the disappearance rate of MPL declines more slowly at subsequent periods. However, considerably more data would be required to validate this suggestion.

Surgical removal of the fetus (fetectomy) was carried out on three pregnant monkeys each on the 80th, 120th, and 150th days of gestation. The placentas of all animals were retained in vivo until surgically delivered from 15 to 62 days later. In each group of three animals,

A: at 80 days

%MPL of control

●----● N gestation
●——● Fetectomy

B: at 120 days
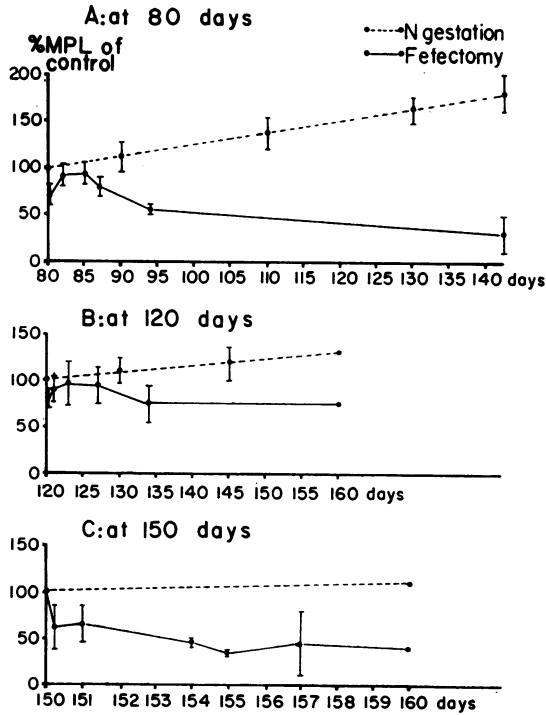
C: at 150 days

FIGURE 8 The MPL concentration in pregnant monkeys fetectomized (A) at 80 days, (B) at 120 days, and (C) at 150 days of gestation are compared with the expected MPL concentration in normal pregnancy at comparable periods of gestation. In each group A, B, and C, fetectomy was carried out on three monkeys and the results were plotted (———) as a mean percentage of the control value ±1 SD. The control value was equal to 100% and was derived from the mean of the MPL concentrations before surgery. The broken line represents the expected rise in normal pregnancy, obtained from Fig. 6, expressed as a percentage of the initial value which was the mean MPL concentration of normal monkeys at 80, 120, and 150 days of gestation.

MPL concentrations were measured before and every 15 min after fetectomy for the first 2 hr; then, every hour for 6 hr; then, every day for 1 wk; and finally, at weekly intervals. Fig. 8 compares the MPL concentration in monkeys after fetectomy at days 80, 120, and 150 with the expected MPL concentration in normal pregnancy as obtained from Fig. 6 at the comparable periods of gestation. In the experimental group a mean of the initial values of MPL determined in three or four samples taken before surgery was assumed to be equal to 100%. In the controls, the mean MPL concentration at the time of fetectomy was also assumed to be 100%, and the expected rise in MPL was expressed as a percentage of the initial value. In all three groups of monkeys the pattern of MPL concentrations after fetectomy is similar, namely, a decrease in MPL concentrations by 25% of the control values occurs in the first 6 hr after fetectomy, followed by a return towards normal 48 hr

later, and finally by a slow progressive decline below normal after 4 or 5 days. The greatest change occurred among monkeys undergoing fetectomy at day 80 and followed for 62 days. MPL concentrations in these animals were only 18% of the levels expected for a gestational period of 142 days. Thus, after fetectomy, MPL concentrations tend to stabilize at lower levels than those occurring in normal pregnancy and fail to show the expected increase as pregnancy advances.

Blocks of tissue were taken from the placentas at the time of their delivery at the second operation. The histological appearance of the tissue revealed intact structures in both primary and secondary placental discs of eight monkeys. The ninth monkey was cachectic at the time of the second operation due to a subcutaneous infection at the incision.
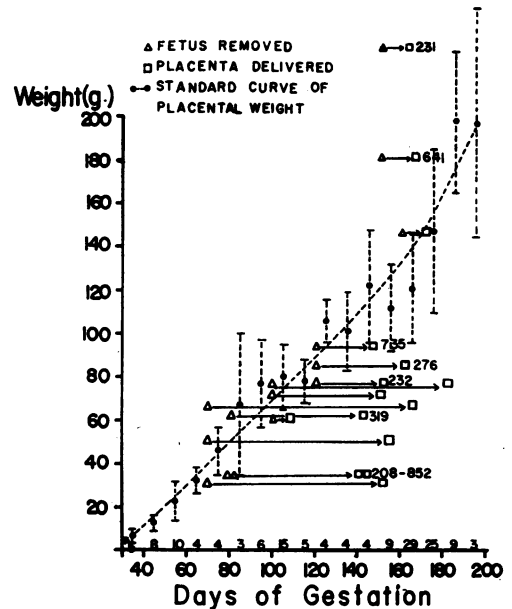

FIGURE 9 The broken line and vertical bars indicate the mean ±SD of the placental weight vs. the days of gestation determined in a series of normal pregnancies. The numbers immediately above the *x* axis indicate the number of placentas weighed at each period. (Reproduced from Van Wagenen, G., and H. R. Catchpole. 1965. *Amer. J. Phys. Anthropol.* **23**: 23–34, with the permission of the publisher and author.) The squares represent the weight of the placentas which were removed at the time of the second operation in the present study. The length of the horizontal line indicates the duration of time which the placenta was retained after fetectomy until the second operation. The triangles indicate the estimated placental weight for the period of gestation when the fetus was removed. The squares followed by numbers are our data and those without numbers are derived from the data of Van Wagenen and Catchpole. The numbers following the squares identify the individual monkeys. The final weight falls well into the normal range for placental weights for the period when fetectomy was carried out. The data show clearly that in the absence of the fetus no placental growth occurred.

TABLE I

Concentration of MPL, HPL, and HCG in Placental Extracts and 24 hr Incubation Media

Hormone	Tissue placental extract	Medium placental extract
	μg/g (dry weight)	μg/g (dry weight)
MPL	330	190
HPL	3000	3500
HCG*	12	12

* The concentration of HCG is expressed in terms of human luteinizing hormone standard (potency of 4 NIH LH U/mg.

A study of placental weight after fetectomy at different stages of pregnancy is presented in Fig. 9. The weights of the retained placentas which were removed at the time of the second operation are compared to a standard curve of placental weights at different stages of pregnancy. Without exception the placental weights failed to increase in the normal fashion in the monkeys from which the fetus had been removed. Our findings are compared with those of Van Wagenen and Catchpole (12) who also failed to observe an increase in placental weight in fetectomized animals.

The MPL concentration was measured in the peripheral blood of six monkeys before and every 30 min after the digital abruption of the secondary placental disc of the rhesus monkey (Fig. 10). A dramatic fall in the MPL concentration was observed within the 1st hour. This decline continued until the concentration reached approximately 50% of the initial concentration at the 6th hour. It may be noted that the secondary placental disc among those animals ranged in size from 30–50% of the total placental weight (weight of primary plus secondary discs).

Six pregnant monkeys were operated upon after the 100th day of gestation. Blood samples were taken before
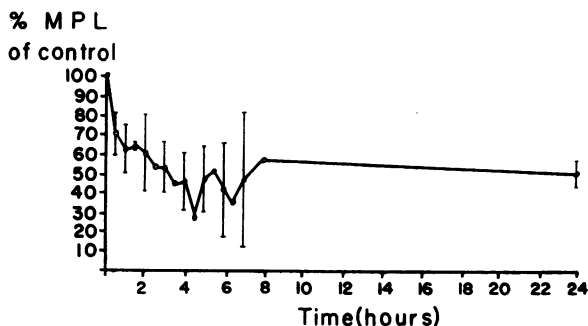

% MPL of control

Time(hours)

Figure 10 Abruptio placenta was induced in six pregnant monkeys. The mean of serum MPL concentration obtained before surgery was assumed to be 100%. The line expresses the mean MPL concentration as a percentage of the control value ±1 sd vs. the time after abruptio placenta.

% MPL of control

o----o control
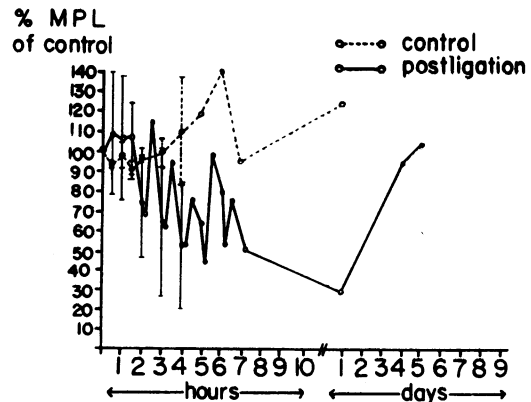o——o postligation

←——hours——→ ←——days——→

Figure 11 Six pregnant monkeys were operated upon after the 100th day of gestation. Four animals underwent ligation of the interplacental vessels, whereas in two control animals the fetus was temporarily withdrawn from the uterus. Serum MPL concentrations were determined before and after surgery and expressed as a percentage of the mean initial value which was assumed to be equal to 100%. The broken line indicates the random variation in MPL concentration in the control animals; the solid line shows that an over-all decrease in the concentration of MPL occurred after ligation of the interplacental vessels.

and every 30 min after ligation of the interplacental vessels (umbilical vessels leading to the secondary placental disc) in four animals. In two further control animals the fetus was temporarily withdrawn from the uterus and blood samples were taken before and every 30 min after the fetus was restored to the uterus. The MPL concentrations in the two groups are compared in Fig. 11. Among the control animals a random fluctuation in MPL concentration occurs whereas among the animals in which the interplacental vessels are ligated an over-all decrease in the concentration of MPL is seen.

The marked differences in MPL and HPL concentrations in placental extracts are indicated in Table I. The concentration of MPL in placental tissue is approximately 1/10 that of HPL. In the incubation medium even less MPL is present; however, the amount of HCG in both tissue and medium is much less than that of either MPL or HPL.

DISCUSSION

MPL is more closely allied to HGH than to HPL immunologically (4). Grant, Kaplan, and Grumbach (5), using a radioimmunoassay for HPL, reported that HGH and MPL behaved in a similar fashion while MPL and HPL reacted very differently in an HGH assay. There appears to be a considerable immunologic difference between MPL and HPL; unlike the close similarity of human and simian growth hormone (13, 14). It is for this reason that a radioimmunoassay for HPL is generally unsuitable for measuring MPL concentrations.

The maternal serum concentrations of MPL and HPL are very similar; the mean for HPL at term has been reported to range from 3 to 10 μg/ml (15–17). In our study MPL concentrations range from 3 to 7 μg/ml. MPL and HPL fail to cross the placental barrier because the concentration of each in cord blood is less than 1% of the maternal MPL or HPL concentration. The endogenous half-lives of MPL and HPL calculated after the removal of the placenta are also very similar. Kaplan, Gurpide, Sciarra, and Grumbach (18) reported a biphasic disappearance of HPL with the half-life of the first phase equal to 12 min compared to an estimated 20 min for MPL. The second phase of HPL had a half-life of 75 min, whereas in the case of MPL insufficient data are available for an accurate assessment of a second phase. From the half-life, plasma concentration, and volume of distribution of MPL and HPL we can estimate that the production rate of MPL is 0.36 g/day compared to 1.0 g/day for HPL. These calculations assume that the volume of distribution of MPL is similar to HPL (10% of body weight), that the average weight of a monkey is 10 kg, and that turnover is monoexponential. In view of the lower tissue content of MPL compared to HPL, turnover of the tissue pool of MPL is considerably greater than that of HPL. Because the secretion of MPL and HPL is similar in many ways it appears likely that MPL is a very useful model for studying factors which regulate the secretion of HPL.

An increasing number of reports have attempted to define the usefulness of measuring HPL concentrations in pregnancy as an index of placental function (17, 19, 20). It was felt that monitoring HPL concentrations might assist the obstetrician to identify fetuses at risk. Our studies on MPL suggest that this is unlikely to be the case. Even when the fetus is removed at various stages of pregnancy, the immediate fall in MPL concentrations is only modest, ranging from 10 to 20%. Only when the secondary placental disc is displaced as in the experiments where an experimental abruptio placenta is induced does a drastic fall in MPL concentration occur. These data suggest that a major compromise in placental function is required before a major change in MPL concentration is observed. However, the concentration of MPL seems to be influenced to a considerable degree by changes in maternal placental perfusion as shown by the fact that when the maternal aorta was constricted, resulting in a decreased intervillous space perfusion, a prompt decrease in MPL concentration in maternal blood was observed (unpublished observations).

The failure of MPL concentrations to increase in the absence of the fetus poses several interesting questions. Was the lack of increase simply a reflection of the arrest in placental development or does the fetus exert a spe-cific role in regulating MPL secretion? Presently available data suggest that placental growth is dependent upon normal fetal growth. In our studies no evidence of a placental weight increase after fetectomy was found in contrast to the study by Behrman, Parer, and de-Lannoy (21) which suggested that placental weight gain continued if the fetectomy was carried out after 120 days of gestation. Rather, our results agree with the findings of Van Wagenen and Catchpole (12) who reported that placental weight remained constant after fetectomy.

Histological and ultrastructural changes in rhesus monkey placenta after interruption of the fetal placental circulation by fetectomy or interplacental vessel ligation have been studied by Panigel and Myers (22). These authors described an early reduction and disappearance of the cytotrophoblast after interruption of the fetal villous blood circulation, suggesting that a continued supply of some humoral or cellular constituent from the developing fetus is required for its continued maintenance. On the other hand, the presence of abundant ergastoplasmic formation (endoplasmic reticulum) in the syncytiotrophoblast for up to 2 months after fetectomy or fetal vessel ligation indicates that the morphologic components required for protein synthesis, including placental hormones, are present in the retained placenta. These histological findings in monkey placentas correlate well with the placental changes described after fetal death in the human (23).

The present studies suggest that the synthesis and secretion of MPL and HPL are similar, and therefore, studies of MPL may prove helpful in elucidating the role of HPL in pregnancy.

ACKNOWLEDGMENTS

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