pithecus monkeys which had been held in captivity. In contrast, no definitely positive reactions were obtained in similar tests of 45 *M. rhesus* sera obtained at time of capture. That the rhesus monkey is susceptible to infection with this group of viruses is evident from the several reports describing typical inclusions in both salivary glands and kidney, and the susceptibility of rhesus testis cultures to the Cercopithecus isolates.

Since Cercopithecus kidney cultures are extensively used in some parts of the world for poliomyelitis vaccine production, and are being used increasingly for experimental work, description of indigenous agents in these cultures is of importance. The cytomegaloviruses described here may be of particular pertinence because of their ability to infect human cells, and the relative difficulty of their detection. The Cercopithecus cytomegaloviruses did not resemble the agents previously described in AGMK by Malherbe and Harwin(16).

Summary. Two similar strains of virus were recovered from Cercopithecus monkey salivary gland tissue and kidney tissue culture. The agents possessed the biological characteristics of cytomegaloviruses, and shared CF antigens with a human cytomegalovirus. They are unique among cytomegaloviruses in their ability to grow in heterologous (human) tissue cultures. Antibodies were common in Cercopithecus held in captivity but were not found in freshly captured rhesus monkeys.

1. Smith, M. G., *Progr. med. Virol.*, 1959, v2, 171 (Karger, Basel/New York).

2. Weller, T. H., Hanshaw, J. B., *N. Engl. J. Med.*, 1962, v266, 1233.

3. Smith, M. G., PROC. SOC. EXP. BIOL. AND MED., 1954, v86, 435.

4. Hartley, J. W., Doctoral Dissertation, 1957 (George Washington Univ., Washington, D.C.).

5. Hartley, J. W., Rowe, W. P., Huebner, R. J., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 281.

6. Kuttner, A. G., Wang, S. H., *J. Exp. Med.*, 1934, v60, 773.

7. Rector, E. J., Rector, L. E., *Am. J. Path.*, 1934, v10, 639.

8. Hahnemann, R. T., Williams, F. P., Fite, G. L., *J. Am. Vet. Med. Assn.*, 1960, v137, 161.

9. Raynaud, J., Raynaud, A., *Ann. Inst. Past.*, 1947, v73, 174.

10. Vogel, F. S., Pinkerton, H., *A.M.A. Arch. Path.*, 1935, v60, 281.

11. Cowdry, E. V., Scott, G. H., *Am. J. Path.*, 1935, v11, 659.

12. Hindle, E., Stevenson, A. C., *Tr. Roy. Soc. Trop. Med. Hyg.*, 1929, v23, 327.

13. Cowdry, E. V., Scott, G. H., *Am. J. Path.*, 1935, v11, 647.

14. Halonen, P., Huebner, R. J., Turner, H. C., PROC. SOC. EXP. BIOL. AND MED., 1958, v97, 530.

15. Rowe, W. P., Hartley, J. W., Waterman, S., Turner, H. C., Huebner, R. J., *ibid.*, 1956, v92, 418.

16. Malherbe, H., Harwin, R., *Brit. J. Exp. Path.*, 1957, v38, 539.

Received December 3, 1962. P.S.E.B.M., 1963, v112.

Vitamin A Ester and Alcohol of Rat Liver During Pregnancy.* (28116)

WARREN N. DANNENBURG, DIRK S. DIXON AND RICHARD L. BURT

Department of Obstetrics and Gynecology, Bowman Gray School of Medicine, Wake Forest College, and North Carolina Baptist Hospital, Winston-Salem

The plasma level of Vit. A in humans(1) and rats(2) during pregnancy is less than that found in non-pregnant and puerperal animals. In addition, the concentration of Vit. A in the liver of pregnant animals is reported to be lower than non-pregnant values(2,3,4). Glover *et al.*(5) showed a proportional relationship between the Vit. A alcohol of liver and that found in plasma, but subsequent investigators (6,7,8) were unable to confirm this observation. Thus the present study was undertaken to determine if any relationship exists between the alcohol and esterified forms of Vit. A in liver and plasma Vit. A during pregnancy.

* This investigation was supported by U. S. Public Health Service grant from Nat. Inst. of Arthritis and Metab. Dis.

TABLE I. Means and Standard Deviations of Vit. A Expressed as μg per Gram of Liver, μg per Total Liver, and μg per 100 ml Plasma for Non-Pregnant, Pregnant, and Puerperal Rats.

Group	No. of rats	Body wt (g)	Liver wt (g)	Vitamin A μg/g liver Total	Ester	Alcohol	Vitamin A μg/total liver Total	Ester	Alcohol	Vitamin A μg/100 ml plasma
Non-pregnant	11	160	6.1	80 ± 9*	62 ± 10	18 ± 6	488	378	110	41 ± 8
Pregnant	12	248	8.7	110 ± 26	86 ± 24	25 ± 7	957	748	148	26 ± 9
Postpartum	5	213	9.2	111 ± 16	93 ± 19	18 ± 7	1021	856	166	38 ±12

* Standard deviation.

Materials and methods. Pregnant albino rats of the Sprague-Dawley strain were obtained from the Holtzman Co., Madison, Wis. 2 days after being sperm positive. Non-pregnant control and sperm positive rats were shipped at the same time so both groups would be subjected to the same conditions during transportation. When the animals were received, they were randomized, placed in cages, and subjected to a 15 hour day and 9 hour night period by the use of artificial lighting in order to reduce the differences in the amount of food consumed. Rats were fed and watered *ad libitum*. The ration used was a rat diet manufactured by Dietrich and Gambrill Inc., Frederick, Md.

Fasted non-pregnant and pregnant (17th gestation day) rats were selected at random and sacrificed on the same day, while puerperal rats were sacrificed on the second postpartum day. The animals were anesthetized by an intraperitoneal injection of sodium pentobarbital and bled from the aorta into heparinized tubes according to the procedure of Lushbough and Moline(9). After collection of the blood, the liver was removed, blotted between filter paper, weighed, wrapped in parafilm and immediately placed in a freezer at −20°C until analyses could be made.. After extracting either 2 ml plasma(10) or a 400-600 mg portion of the left lower lobe of the liver(11), the extract was assayed for total Vit. A according to Kimble(10) using standards as previously described(1).

Vit. A alcohol and ester were separated on an alumina column employing a modification of the procedure described by Eden(12). Adsorption alumina (Fisher Scientific Co. 80-200 mesh) was prepared(13) and 0.7 g placed in a column 150 mm long with an I.D. of 8 mm. The alumina was washed with petroleum ether (30-60°C) and stirred with a glass rod to remove air pockets. Two milliliters of the Vit. A extract was taken to dryness(11), dissolved in 2 ml of petroleum ether, and 1 ml placed on the column. Vit. A ester was eluted with 10 ml of 4% acetone, Vit. A alcohol with 10 ml of 50% acetone in petroleum ether, the eluates collected and evaporated in Evelyn tubes under reduced pressure(11), and assayed as described for total Vit. A. Recoveries of Vit. A solutions of known concentrations from the alumina were found to be 98% for Vit. A palmitate and 96% for Vit. A alcohol. Although the recovery of the alcohol was considered satisfactory, values were determined by difference (5). Results are reported as micrograms of total Vit. A per 100 ml plasma and as micrograms of Vit. A ester and alcohol per gram and per total liver. Significant differences between means were determined by the Fisher "t" test, and means were considered significantly different if $P<.05$(14).

Results. Levels for Vit. A in plasma and liver with their standard deviations are shown for the 3 groups studied in Table I. The data show that the mean plasma level for Vit. A during pregnancy was lower than that found in either the non-pregnant ($P<.001$) or puerperal ($P<.05$) animals, and that puerperal plasma Vit. A levels were not significantly different from non-pregnant values.

When total and esterified Vit. A per gram of liver in non-pregnant rats is compared to those values in the pregnant and puerperal groups, it was found that the mean differences were higher for the latter 2 groups and significant at the 1% and 0.1% levels, respectively. However, no statistical differences in these liver components were observed between pregnant and postpartum animals.

Vit. A alcohol per gram of liver in the pregnant rats was significantly higher than those values in the non-pregnant ($P<.01$) and puerperal rats ($P<.05$) and as in plasma, no differences were found between non-pregnant and puerperal animals.

Absolute amounts of Vit. A were determined on a total liver basis and it is seen (Table I) that all fractions increased with an increase in the liver weight. Of interest were results obtained from an additional group consisting of 4 non-pregnant rats having an average body weight (222 g) similar to those of the pregnant and postpartum groups in Table I. In these animals, the average liver weight was 6.1 g, which was the same as observed in non-pregnant rats weighing less. Analyses of these livers showed averages of 189 μg, 168 μg, and 20 μg, of total, ester, and alcohol Vit. A per gram of liver, respectively. Finally, Vit. A alcohol in liver was plotted against its corresponding value in plasma for each rat in the groups studied and no linear relationship was apparent.

Discussion. Though plasma Vit. A in fasted rats has been reported to be predominantly in the alcohol form(5), depletion studies(8,15) indicate that the ester is present in considerable amounts. Consequently, plasma Vit. A levels cannot be accurately expressed as the alcohol and are reported as total plasma Vit. A. The changes in plasma Vit. A occurring in pregnancy as shown in Table I agree with the report of Henry *et al.*(2) in that values are decreased in pregnancy and return to prepregnant values by the second postpartum day. This observation has also been made in humans(1).

Though the evidence is not unequivocal, the data indicate an inverse relationship between Vit. A alcohol in the liver and plasma Vit. A during pregnancy. This relationship is not a proportional one as reported by Glover *et al.*(5) and McGillivray(15), nor is it linear. However, the data are consistent with the close relationship between Vit. A alcohol in the liver and plasma Vit. A and show that changes in plasma levels during pregnancy are associated with changes in the alcohol form in the liver that differ significantly from non-pregnant values. The decrease in liver Vit. A alcohol to prepregnant levels following parturition parallels the increase observed in plasma and offers further evidence of this relationship.

Liver Vit. A calculated on a gram basis, appears to be a function of the body weight (16,17) and not the physiological condition (4) of the animal. This increase with body weight is associated with the storage or ester form of Vit. A(16,17) and a comparison of these values found in the livers of the non-pregnant, pregnant, and puerperal rats in Table I shows such an increase. The disparity of this observation between the higher ester values found in the additional group of non-pregnant rats weighing 220 g and the pregnant and puerperal rats having similar body weights (Table I) is apparent, but may be explained by the fact that the weight increase associated with pregnancy is temporary and probably does not reflect comparable changes in Vit. A ester. It then appears that total liver Vit. A levels in pregnancy are influenced by prepregnant body weights as well as increased weight of the hypertrophied liver, and due to these variables, changes based on total liver would be difficult to detect. On a unit basis, however, changes in liver Vit. A alcohol and plasma Vit. A during pregnancy appear to be independent of these variables and must be a function of something besides body weight and Vit. A ester levels.

High and Wilson(8) suggested that plasma Vit. A is controlled by a mechanism apart from the concentrations of Vit. A ester and alcohol in the liver. However, this appears to be only partially correct in pregnancy as the data presented indicate that plasma Vit. A is not independent of liver Vit. A alcohol. It is likely that the observations reported by Glover *et al.*(5) and McGillivray(15) and those presented here have the same basic mechanism, the difference being related to physiological effects resulting from pregnancy. Thus a direct relationship between the proportion of Vit. A alcohol of liver and plasma Vit. A is observed with partial hepatectomy, Vit. A supplement or hormone administration(5, 15), while an indirect relationship prevails during pregnancy.

It was previously suggested that a close as-

sociation existed between Vit. A and nonesterified fatty acid (FFA) levels(1). Such an association is not without basis as FFA (18) and Vit. A(19) are reported to be bound to β-lipoprotein during transport, and it is this lipoproten which has been reported to increase during pregnancy(20). In view of the report of Goodman and Shafrir(18) that the binding sites on β-lipoprotein showed affinities for FFA as great as that of albumin, it is possible that the increased plasma lipids found during pregnancy(21) occupy sites normally occupied by Vit. A. Though it is only speculative, it appears that the low levels of plasma Vit. A in pregnancy may be related to that of transport and are secondary to the hyperlipidemia of pregnancy in that available binding sites for Vit. A alcohol are occupied by FFA resulting in accumulation of Vit. A alcohol in the liver and a decrease in plasma Vit. A. Such physiological changes in Vit. A taking place in pregnant animals do not represent a deficiency of the vitamin as evidenced by the high levels found in the liver. However, if based on plasma levels, an incipient deficiency might be suspected.

Summary. During pregnancy plasma Vit. A decreased and liver Vit. A alcohol increased but both returned to prepregnant values by the second postpartum day. The data indicate that an inverse relationship exists in pregnancy between plasma Vit. A and liver Vit. A alcohol that is unrelated to esterified Vit. A, body weight, and liver hypertrophy. It was suggested that changes in plasma Vit. A levels were related to transport and secondary to the hyperlipidemia of pregnancy. A possible mechanism involved is discussed.

The authors wish to thank Miss Mary Brown and Mrs. Sue Hardin for technical assistance.

1. Pulliam, R. P., Dannenburg, W. N., Burt, R. L., Leake, N. H., PROC. SOC. EXP BIOL. AND MED., 1962, v109, 913.

2. Henry, K. M., Kon, S. K., Mawson, E. H., Stanier, J. E., Thompson, S. Y., *Brit. J. Nutr.*, 1949, v3, 301.

3. Wolff, L. K., *Lancet*, 1932, v2, 617.

4. Morgan, A. F., White, C. E., *J. Am. Diet. Assn.*, 1950, v26, 569.

5. Glover, J., Goodwin, T. W., Morton, R. A., *Biochem. J.*, 1947, v41, 97.

6. Caster, W. O., Mickelson, O., *Am. J. Clin. Nutr.*, 1955, v3, 409.

7. Ganguly, J., Krinksy, N. I., Mehl, J. W., Deuel, H. J., Jr., *Biochem. J.*, 1951, v54, 177.

8. High, E. G., Wilson, S. S., *Arch. Biochem. Biophys.*, 1956, v62, 163.

9. Lushbough, C. H., Moline, S. W., *Proc. Animal Care Panel*, 1961, v11, 305.

10. Kimble, M. S., *J. Lab. Clin. Med.*, 1939, v24, 1055.

11. Gade, E. T., Kadlec, J. D., *J. Agric. Food Chem.*, 1956, v4, 426.

12. Eden, E., *Biochem. J.*, 1950, v46, 259.

13. Parrish, D. B., *J. Assn. Offic. Agr. Chemists*, 1959, v42, 520.

14. Snedecor, G. W., *Statistical Methods*, Iowa State Coll. Press, Ames, 5th ed., 1959.

15. McGillivray, W. A., *Brit. J. Nutr.*, 1961, v15, 305.

16. Dowling, J. E., Wald, G., *Proc. Nat. Acad. Sci.*, 1958, v44, 648.

17. Guerrant, N. B., *J. Nutr.*, 1949, v37, 37.

18. Goodman, D. S., Shafrir, E., *J. Am. Chem. Soc.*, 1959, v81, 364.

19. Onecly, J. L., Gurd, F. R. N., Melir, M., *J. Am. Chem. Soc.*, 1950, v72, 458.

20. DeAlvarez, R. R., Gaiser, D. F., Simkins, D. M., Smith, E. K., Brotvold, G. E., *Am. J. Obst. and Gynec.*, 1959, v77, 743.

21. Boyd, E. M., *J. Clin. Invest.*, 1934, v13, 347.

Received December 3, 1962. P.S.E.B.M., 1963, v112.