TABLE IV. Specificity of Hemagglutinins Found in Tissue of RV-infected Hamster.

Guinea pig serum	HA units of RV-infected liver susp.	Dilutions of guinea pig serum 20	40	80	160	320	640	1280	2560	5120
Pre-inoc.	16	0	+	+	+	+	+	ND	ND	ND
	8	0	0	+	+	+	+	"	"	"
	4	0	0	0	+	+	+	"	"	"
3 wk post-inoc.*	16	0	0	0	0	0	0	+	+	+
	8	0	0	0	0	0	0	0	+	+
	4	0	0	0	0	0	0	0	0	+

* Guinea pig immunized with RV-infected rat embryo tissue culture fluid.

have shown that polyoma virus reaches peak titers in 4 to 6 days after inoculation, but titers of infective virus are low, hemagglutinins fail to appear and there is no immediate disease or subsequent dwarfism. Neoplasms are the characteristic signs of polyoma infection in infected hamsters. The multiplication of polyoma virus in suckling mice (4) follows patterns which are closer to those described above for rat virus in suckling hamsters. Thus Rowe *et al.*, encountered infective virus in titers of 10^{-6} and 10^{-7} in kidneys and livers of infected animals and these were associated with presence of hemagglutinins. Polyoma virus was also demonstrable in both blood and urine. The fact that this agent can induce dwarfism associated with defective incisor teeth in mice is an additional point of similarity with RV (2).

Summary. 1) Rat virus (RV) can induce 3 different conditions in suckling hamsters according to dose of virus and age of the animal at time of inoculation. These conditions are (a) a fatal disease in sucklings 1 to 4 days of age; (b) a stunted growth in those surviving a minimal dose, and (c) a latent infection of older sucklings. 2) Spontaneous transmission of RV can take place from inoculated to uninoculated hamsters. 3) Infected sucklings have infective virus in high titer in livers, kidneys and other organs as well as in lesser amounts in blood and urine. Specimens with the highest infectivity titers have also had considerable amounts of hemagglutination activity. 4) Rat virus has been carried through 20 passages in suckling hamsters. Intracerebral, intranasal, subcutaneous and intraperitoneal routes of inoculation have all been effective in inducing acute disease.

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Studies of Placental Morphogenesis I. Radioautographic Studies of Human Placenta Utilizing Tritiated Thymidine.* (26490)

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The manner in which the syncytiotrophoblast takes origin and is maintained has occupied the attention of many investigators interested in the placenta. This paper presents preliminary data from investigations of this problem.

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Method. Fresh sterile human placental tissue was obtained from 4 therapeutic abortions ranging in gestational age from 14 to

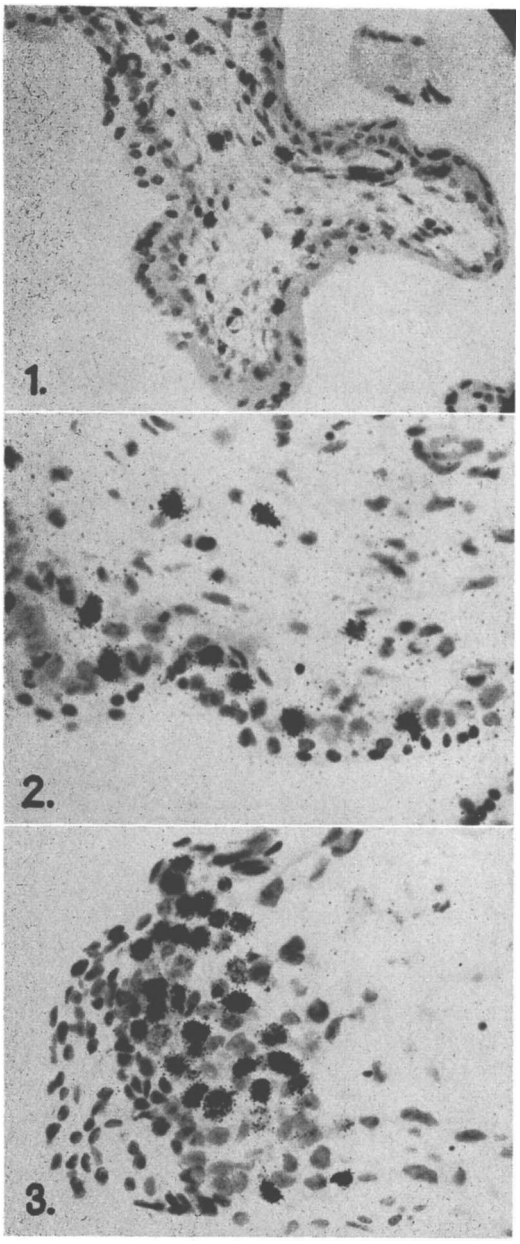

FIG. 1. Radioautograph of human placenta. Note labeled nuclei among cells of cytotrophoblast, villous mesenchyme, and vascular endothelium.

FIG. 2. Radioautograph of human placenta with many labeled nuclei among cells of cytotrophoblastic layer but no labeled nuclei among syncytiotrophoblastic cells.

FIG. 3. Radioautograph of human placenta with intense activity among nuclei of cytotrophoblastic column but no labeled nuclei in peripheral syncytiotrophoblast.

18 weeks. Full thicknesses of placental tissue were placed in Puck's F4FC tissue culture media to which had been added tritiated thymidine of 1.9 μc per millimole specific activity at concentrations ranging from 1 to 12 μc per milliliter of media. The cultures were maintained at 37 $\pm$ 0.2°C with air as the gas phase and pH was adjusted daily to 7.2. Samples of the placental tissue were removed from incubation, washed in balanced salt solution and fixed in neutral buffered formalin at intervals ranging from 1 to 72 hours. The tissues were prepared for sectioning, cut at 6 mu, and mounted on glass slides. The sections were deparaffinized in xylol, hydrated and dipped in undiluted Kodak liquid emulsion type NTB 3, and exposed at 4°C for 3 to 4 weeks in bakelite boxes containing Drierite. They were then developed in Kodak *D-19* developer, stained with Harris' hematoxylin. coverslipped, and examined microscopically.

Results. In more than 200 slides examined, each containing from 8 to 12 sections, unequivocal evidence of thymidine uptake by syncytiotrophoblastic nuclei was not encountered. There was abundant activity in nuclei of the villous mesenchyme and vascular endothelium as well as the Langhans cytotrophoblast layer (Fig. 1, 2). There was particularly heavy activity in the nuclei of the cytotrophoblastic columns (Fig. 3).

Discussion. The problem of how the syntiotrophoblast takes origin has been discussed by Hertig *et al.*(3) Wislocki and Bennett(5) and Wimsatt(4), all of whom proposed an amitotic form of division as one hypothesis to account for the quantitative increase in the syncytium without morphologically distinguishable mitotic activity. Hamilton and Boyd(2) also offered amitotic division as a possible mechanism for the origin of the syncytiotrophoblast.

Our failure to demonstrate thymidine uptake by syncytial nuclei argues strongly against amitotic division in the syncytiotrophoblast, at least in the human placenta under the conditions of our experiments. It may be argued that amitotic divisions could occur in absence of new DNA synthesis and

hence without thymidine uptake but this is unlikely in the case of human syncytiotrophoblast in view of the near-diploid DNA valves found by Galton(1) in these nuclei using microspectrophotometry.

On the basis of these experiments it now seems likely that the syncytiotrophoblast is a mitotic end stage that is continually derived from another cell type, possibly cytotrophoblast. Experiments are under way to investigate this possibility and to extend our findings to other species and other experimental conditions.

Summary. Portions of human placental tissue have been studied by radioautography utilizing tritiated thymidine. Thymidine uptake could be demonstrated in cytotrophoblast, villous mesenchyme and vascular endothelium but not in the syncytiotrophoblast. This finding suggests that amitotic division of the syncytiotrophoblast does not play a prominent role in the derivation of this cellular layer in the human placenta of 14-18 weeks gestational age.

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Effects of Time and Steroid Concentration on Binding of C^{14}-Estrogens in Human Plasma.* (26491)

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We have reported(1-3) on the distribution of radioactive steroids and their metabolites among the various plasma protein fractions following intravenous injection of C^{14}-steroids into human subjects. Thus, it was shown that most unconjugated and conjugated steroids and their metabolites were bound principally to the plasma fractions IV-1 and V. In the case of C^{14}-estrone and C^{14}-estradiol the bloods were drawn 20 and 45 minutes after injection, respectively. This study deals with the effects of time of blood withrawal and prior administration of large amounts of non-radioactive steroids, either identical to or different from the radioactive steroids, on distribution of radioactivity among the fractionated plasma proteins.

Experimental. The materials, methods and experimental procedures were similar to those previously reported(1,2) with the following exceptions: 1) *ca.* 250 ml of blood was withdrawn 30 minutes after injection and an equal volume 90-150 minutes later and 2) two subjects, in addition to receiving radioactive steroid, were infused a second time with the same radioactive steroid plus 50 mg of non-radioactive steroid. The solution for infusion was prepared by dissolving 50 mg of crystalline U.S.P. steroid in 10 ml of ethanol, which was added dropwise with stirring to 240 ml of 1% HSA (human serum albumin) resulting in the appearance of a very fine microcrystalline suspension of the steroid. This solution was infused intravenously over 30 minutes. The C^{14}-steroid (2.7 μc/mg) dissolved in 50 ml of 4% ethanol in saline, was injected intravenously over a period of 2-3 minutes through the tubing halfway through the infusion. The timing commenced with injection of the radioactive steroid.

Before discussing the results, it may be worthwhile to comment on the state of the carrier and radioactive steroids injected in

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