crease in 0.5 M saline soluble, insoluble and total collagen. Since less than 5% of total soluble nitrogen is not protein, this constancy of dermal nitrogen means that non-collagenous dermal proteins or glycoporteins are being synthesized. Therefore insoluble collagen is specifically decreased in response to local inflammation. It is suggested that this distant dermal collagen response to local inflammation results from enzymatic collagenolysis which stimulates the attempted synthesis of replacement collagen. A steady state with respect to collagen anabolism and catabolism could then be established in which, by reason of the relative rates of synthesis and degradation, the skin would contain subnormal amounts of 0.5M saline soluble and insoluble collagen and supernormal concentrations of pro-collagen.

1. Houck, J. C., Jacob, R. A., PROC. SOC. EXP. BIOL. AND MED., 1958, v98, 655.
2. Jacob, R. A., Houck, J. C., *Surg. Gyn and Obst.*, 1959, v109, 85.
3. Houck, J. C., Jacob, R. A., *Am. Surg.*, 1959, v25, 344.
4. Houck, J. C., Jacob, R. A., Maengwyn-Davies, G. D., *J. Clin. Invest.*, 1960, v39, 1758.
5. Houck, J. C., Jacob, R. A., PROC. SOC. EXP. BIOL. AND MED., 1960, v105, 324.
6. Blix, G., *Acta. Chem. Scand.*, 1948, v2, 467.
7. Martin, C. J., Axelrod, A. E., PROC. SOC. EXP. BIOL. AND MED., 1953, v83, 461.
8. Houck, J. C., Patel, Y. M., *ibid.*, 1959, v102, 421.
9. Houck, J. C., Jacob, R. A., *J. Invest. Dermatol.*, 1961, in press.

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Placental Transfer of Fluorine in the Human Fetus.* (26266)

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Recent studies have established certain relationships between daily fluorine intake by pregnant women and fluorine content of the maternal blood, placental tissue and cord blood(1,2,3,4). Gardner *et al.*(1) found significantly higher fluorine values in maternal blood and placental tissues of pregnant women living in Newburgh, N. Y. where fluorine content of the water is 1 ppm, than in those living in Rochester, N. Y., where the water is fluorine-free. Held(2) reported a marked increase in maternal and cord blood fluorine levels after supplemental fluorine had been given. Feltman and Kosel(3) compared fluorine concentration in cord blood and placental tissue in women drinking fluorine free water with that in women given fluoridated water or fluorine tablets. In both groups placental tissue contained much more fluorine than cord blood. Ziegler(4), using pooled materials, compared fluorine values in placental tissue, maternal blood and cord blood of a small number of women drinking almost fluorine-free water, with those in women given additional fluorine in milk. In the latter group there was a very significant increase of fluorine concentration in maternal blood and placental tissue; in cord blood the value increased only slightly.

The present study shows a similar relationship in a larger number of subjects, each individually investigated.

Materials and methods. Fluorine determinations were carried out on blood samples taken from 25 healthy, pregnant women shortly before their delivery in the obstetrical department of Hadassah-University Hospital, and from 32 healthy, non-pregnant women. All the subjects lived in Jerusalem, which is supplied by drinking water containing 0.55 ppm fluorine(5). Placentas were obtained at normal deliveries occurring at full term. Net weights of the placentas ranged

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TABLE I. Fluorine Concentration in Placental Tissue, Cord and Maternal Blood.*

Material	No. of cases	F value, ppm Mean	Range	Material compared	Significance of difference
1. Placental tissue	25	.15	.27-.07	1:3	$p < .001$
2. Cord blood	25	.11	.25-.03	1:2	$p < .05$
3. Maternal blood	25	.09	.21-.02	2:3	$p = .075$ (none)
4. Non-pregnant women's blood	37	.18	.36-.04	3:4	$p < .001$

* Maternal blood shortly before delivery.

from 330-795 g. In each case the whole placenta, as well as the blood from the cord after its separation, were analyzed for fluorine content. Examination of the whole placenta was preferred to avoid errors due to a possibly unequal distribution of fluorine in placental tissue(3).

After 20-25 cc of blood and placenta were each made alkaline with fluorine-free Ca $(OH)_2$ solution (which served also as fluorine fixative), the samples were evaporated to dryness and ashed at about 400°C. The ashed samples were transferred to the distilling apparatus previously described(6) and the chlorides were precipitated with Ag_2SO_4. Fluorine was then steam distilled in presence of H_2SO_4 at 135°-138°C. In the concentrated distillate fluorine was determined colorimetrically by the color fading reaction of a violet iron salicylate complex, as described by Voegtlin and Hodge(7) and Cremer and Voelker(8). The experimental error of this method for blood and placenta samples is ± 0.03 ppm fluorine.

Results. The results are presented in Table I. Mean fluorine values of the placental tissues were significantly higher than in the cord or maternal blood (0.15 ppm, 0.11 ppm, and 0.09 ppm respectively). Mean fluorine content in cord blood was not significantly higher than in maternal blood. Mean fluorine value in the blood of non-pregnant women was significantly higher than that found in the maternal blood before delivery (0.18 ppm and 0.09 ppm respectively). Placenta-cord blood and placenta-maternal blood correlation coefficients were determined in each case. The correlation coefficient between fluorine concentrations in placental tissue and cord blood was significant (r: + 0.5182). No significant correlation was evident between fluorine concentration in placental tissue and that in maternal blood (r: + 0.1795).

Discussion. The significantly lower fluorine level in maternal blood shortly before delivery, compared with that found in the blood of non-pregnant women, corresponds with the decrease of fluorine concentration occurring in urine and saliva of pregnant women(5,9). The significantly higher level of fluorine in the placenta, as compared with that in the maternal blood, indicates that fluorine accumulates in placental tissue. This agrees with the results of Gardner *et al.*(1), and of Ziegler(4). Fluorine accumulation in the placenta does not appear to be related to the actual fluorine concentration found in the maternal blood shortly before delivery. Maternal blood fluorine concentration depends mainly on fluorine content of food and water, and may be subject to daily variations(10, 11). However, due to the amount of blood (about 20-25 cc) needed to determine such variations, repeated estimations were not performed.

Although there was no significant correlation between fluorine in placental tissue and fluorine in maternal blood, in all the cases studied placental concentration of fluorine was always higher than that of maternal blood(4).

The finding that mean fluorine concentration in the placenta is significantly higher than that in cord blood, and that a positive correlation exists between them, indicates that the placenta allows transfer of fluorine to the fetus. The fluorine is utilized mainly for growth development of the bones and teeth of the fetus(12,13,14).

It may be concluded from the above results that fluorine accumulates in the placenta which, at least in regions supplied by drinking water of about 0.55 ppm fluorine,

presents little obstruction to passage of fluorine from maternal to fetal circulation. The fact that concentrations of fluorine in maternal and cord blood are almost the same was considered by Held(2) to imply that the placenta passively permits fluorine transfer to the fetus. Our finding that fluorine concentration in the placenta is higher than in either maternal or cord blood suggests, however, that the placenta plays a more active role.

Summary. Samples of the blood from pregnant women shortly before delivery, placentas at full term and cord blood, were analyzed for fluorine. All subjects drank from the same water source which contained about 0.55 ppm fluorine. The results suggest that the placenta plays an active role in accumulation of fluorine and its transfer to the fetus.

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1. Gardner, D. F., Smith, F. A., Hodge, H. C., Overton, D. E., Feltman, R., *Science,* 1952, v115, 208.

2. Held, H. R., *Schweiz. med. Wchnschr.,* 1954, v84, 251.

3. Feltman, R., Kosel, J., *Science,* 1955, v122, 560.

4. Ziegler, E., *Mitt. d. naturwiss. Gesellsch., Winterthur,* 1956, v28, 39.

5. Gedalia, I., Brzezinski, A., Bercovici, B., *J. D. Res.,* 1959, v38, 548.

6. Gedalia, I., Yardeni, J., *ibid.,* 1957, v36, 203.

7. Voegtlin, C., Hodge, H. C., *The Pharmacology and Toxicology of Uranium Compounds,* McGraw-Hill, New York, 1951.

8. Cremer, H. D., Voelker, D., *Biochem. Z.* 1953, v324, 89.

9. Lerman, D., Gedalia, I., Rosenzweig, K., Brzezinski, A., *Proc. 6th Congr. ORCA,* 1959, in press.

10. Smith, F. A., Gardner, D. E., Hodge, H. C., *J. D. Res.,* 1950, v29, 596.

11. Shaw, J. H., *Fluoridation as a Public Health Measure,* A.A.A.S., Washington, 1954, p54.

12. Buttner, G., Muhler, J., *J. Dental Res.,* 1958, v37, 326.

13. Ericsson, Y., Ullberg, S., *Proc. 5th Congr. ORCA,* 1958, p200.

14. Brzezinski, A., Bercovici, B., Gedalia, I., *Obst. and Gynec.,* 1960, v15, 329.

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Carbon Dioxide Requirement and Nucleic Acid Metabolism of HeLa and Conjunctival Cells.* (26267)

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This is the third of a series of publications from this Department on the physiological role of carbon dioxide in multiplying human cells *in vitro.* The following are some of the justifications for carrying out such studies: a) while many compounds are known to have arisen through CO_2 fixation in the heterotrophic organisms, the physiological significance of many such reactions remains obscure; b) the importance of CO_2 in the process of differentiation has been reported(1); c) there are speculations that CO_2 may act as a physiological regulator of cell multiplication(2), and d) very few reports are based on study with intact human cells.

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Our first report described CO_2 as an essential for propagation of human cells *in vitro* (3). A subsequent paper demonstrated the role of CO_2 in RNA and protein synthesis as shown by virus formation in human cell culture(4). The present report describes the fixation of $C^{14}O_2$ by human cells and recovery of most of the fixed C^{14} in the nucleic acids and their derivatives. Under the described experimental conditions, the syntheses of nucleic and oxaloacetic acids are the only demonstrable essential CO_2 fixation reactions of these human cells.

Materials and method. Nutrient media. The tris(hydroxymethyl)aminomethane buffered medium was used exclusively, unless