gram (10- by 10-cm usable surface) was prepared, according to the ascending method used by us (*3*) with water as the solvent, in an atmosphere saturated with vapors of acetic acid. The numbers correspond to substances which have been identified by their spots in chromatograms and characterized by physical and chemical properties of the spots or their eluates (*3*). The diagonal has been traced as a curved line, the curving resulting from the accumulation of a substance in the filter paper at the front line of the first chromatography. This substance, which is not entirely removed by repeated washing of the filter paper, slows down the flow of the solvent during the second chromatography. Two horizontal rows of decomposition products at R_f values 0.14 and 0.71 are seen on the chromatogram. They cross the diagonal line at primary spots 2 and 4.

On the basis of this and other chromatograms, as well as on the basis of physical and chemical properties of the eluates, these spots are interpreted as corresponding to the keto and the enol forms of indolepyruvic acid. A secondary spot of substance 4 appears in the trail of substance 2, and vice versa. Secondary spots of substances 1, 8, 6, 7, and 5 appear on the horizontal trails of both 2 and 4 and correspond to decomposition products of these substances. Secondary spot 1*a* appears in the trail of substance 4 only, and secondary spot 3 in that of substance 2 only. Only one vertical trail, that of substance 1, has been traced, because the others were faint and because the chromatogram was made at a time when their significance was not yet well understood. Composite spots such as the cluster near the primary spot of substance 4 and the large spot of substance 8 have been interpreted according to their position in relation to the diagonal and the horizontal rows of spots as well as to their fluorescent colors.

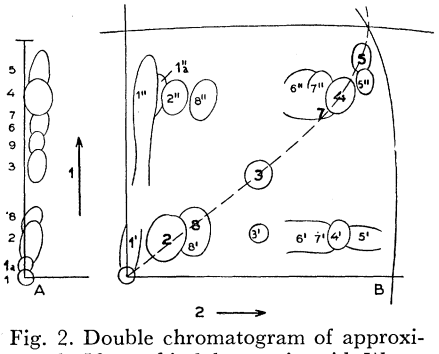

Fig. 2. Double chromatogram of approximately 50 μg of indolepyruvic acid. Water was used as solvent in both directions. The container was saturated with vapors of acetic acid. Ascending chromatography. Primary spots are distributed along the curved diagonal line. Secondary spots marked with a prime sign are from the decomposition products of substance 2, and those marked with a double prime sign are from the decomposition products of substance 4. The keto and the enol forms of indolepyruvic acid have produced, respectively, the lower and the upper row of spots of decomposition products.

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References and Notes

1. This work has been supported in part by grants from the Brazilian Research Council (CNPq) and the Rockefeller Foundation.
2. The sample of indolepyruvic acid was kindly supplied by K. V. Thimann.
3. K. Schwarz, A. P. Nogueira, A. A. Bitancourt, "Spontaneous decomposition of some indole derivatives," in preparation.
* Research fellow, CNPq.

6 May 1957

Effect of Cyanide on Biosynthesis of Ascorbic Acid in vitro

It was observed in this laboratory that when rat liver tissue, homogenized in a Potter-Elvehjem homogenizer with 0.25*M* sucrose, was incubated with certain compounds under suitable conditions, an increase in the ascorbic acid value occurred over that obtained without the added substrate, the ascorbic acid being measured titrimetrically with 2,6-dichlorophenol indophenol. It was, however, noticed that in no case could a net positive synthesis of ascorbic acid be attained; that is to say, the ascorbic acid content after incubation never exceeded that present in the tissue before incubation.

In the search for a means of developing a system in which a net positive synthesis could be realized, it has been found that when rat liver homogenate is incubated with D-glucuronolactone in the presence of potassium cyanide ($5 \times 10^{-2}M$), the ascorbic acid value obtained after incubation is markedly greater than that obtained without the added substrate; the value can, in fact, be 2 to 3 times the original tissue content of ascorbic acid. Without cyanide, no increase in ascorbic acid value has been observed (Table 1). Also, when the concentration of cyanide is below $5 \times 10^{-3}M$, the influence of cyanide on the synthesis is practically nil. On the other hand, an increase in the cyanide concentration above $5 \times 10^{-3}M$ steadily increases the synthesis until a concentration of $5 \times 10^{-2}M$ is reached, when no further enhancement of the synthesis of ascorbic acid is observed. This increase in the ascorbic acid value under the influence of cyanide is not revealed when sodium D-glucuronate is substituted for D-glucuronolactone. Boiled tissue extracts give no positive results under these conditions.

Hassan and Lehninger (*1*) have shown that the enzyme system concerned in this synthesis resides in the supernatant fluid after sedimentation of the nuclear fraction and mitochondria. The aforesaid capacity of liver homogenate to convert D-glucuronolactone into ascorbic acid in the presence of cyanide has also been found to be present almost entirely in this fluid, obtained according to the method of Hogeboom *et al.* (*2*). This synthesis under the influence of cyanide is not further enhanced by the addition of cofactors like diphosphopyridine nucleotide (0.0016*M*), adenosine triphosphate (0.0016*M*), and magnesium chloride (0.004*M*), either separately or conjointly.

The results obtained with cyanide appear, however, to be at variance with those reported by Hassan and Lehninger (*1*), who observed an *inhibition* of the synthesis of ascorbic acid in the presence of 0.01*M* cyanide, although they observed none with $5 \times 10^{-4}M$ cyanide. In a repetition of their experiments it has been observed that, in their system, in which adenosine triphosphate and other cofactors are used, a small increase in ascorbic acid of the order of 0.17 to 0.23 μm is obtained with both the lactone and the sodium salt of glucuronic acid. Even in this system, however, the addition of cyanide ($5 \times 10^{-2}M$) has been found to increase the synthesis to 0.30 to 0.35 μm of ascorbic acid, and this again with the lactone as substrate and not with sodium glucuronate. It is possible that, for the formation of ascor-

Table 1. The effect of potassium cyanide on the *in vitro* synthesis of ascorbic acid. The test system contains: 0.06*M* phosphate buffer (*p*H 7.0); 0.025*M* substrate; tissue concentration 50 mg (or its equivalent of supernatant fluid) per milliliter of incubation mixture; KCN 0.05*M*. Total volume is 5.0 ml, incubated at 37.5°C for 2 hours. Negative figures indicate loss of ascorbic acid on incubation.

Expt.	Substrate	Ascorbic acid (μm) synthesized	
		Without KCN	With KCN
Rat liver homogenate	None	−0.22	0
	D-Glucuronate	−0.24	0
	D-Glucuronolactone	−0.24	+0.77
Rat liver extract (supernatant fluid)	None	−0.20	0
	D-Glucuronate	−0.20	0
	D-Glucuronolactone	−0.20	+0.61

Table 2. The effect of cyanide on the *in vitro* synthesis of "total" ascorbic acid. The conditions are the same as in Table 1, except that the concentration of glucuronolactone was $0.0125M$. In determining "total" ascorbic acid, the metaphosphoric acid extract of the tissue was diluted sufficiently to avoid interference by cyanide, the final cyanide concentration of the diluted extract being $4 \times 10^{-3}M$.

Expt.	Substrate	Ascorbic acid (μm) synthesized	
		Without KCN	With KCN
Rat liver homogenate	None	0	0
	D-Glucuronate	0	0
	D-Glucuronolactone	0	+0.58
Rat liver extract (supernatant fluid)	None	0	0
	D-Glucuronate	0	0
	D-Glucuronolactone	0	+0.31

bic acid, the lactone structure is necessary and that cyanide prevents lactonization of glucuronic acid in the system studied. This might explain the difference in the behavior of the acid and of the lactone in this regard. In looking for the cause of the inhibition by cyanide observed by Hassan and Lehninger (*1*), it has been found that the method of Roe and Kuether (*3*), which was used by them for the determination of "total" ascorbic acid, would give low values for ascorbic acid in the presence of $0.01M$ cyanide. Thus, in a recovery experiment starting with pure L-ascorbic acid, it has been found that in the presence of $0.01M$ cyanide, only 2 to 3 μg out of 27 μg could be estimated by that method, while the ascorbic acid could be correctly estimated in the presence of $5 \times 10^{-4}M$ cyanide.

In order to throw further light on this question, estimations were also made of the "total" ascorbic acid in the present set of experiments (*4*) by Roe and Kuether's method. Without KCN, original values of ascorbic acid could be obtained after the period of incubation. But in experiments involving the use of KCN, the "total" ascorbic acid values, as estimated by that method, were lower than those obtained with the indophenol indicator. Nevertheless, these values were higher than the original values for "total" ascorbic acid in these tissues, showing a net positive synthesis of ascorbic acid from D-glucuronolactone in the presence of cyanide (Table 2).

That cyanide in the afore-described experiments actually facilitates the biosynthesis of ascorbic acid and does not merely act as a stabilizer has been confirmed by experiments in which it has been found that KCN at a concentration of $1 \times 10^{-3}M$ can completely protect (i) pure ascorbic acid, (ii) the ascorbic acid in the tissue, and (iii) pure ascorbic acid added to the tissue, whereas at this concentration no biosynthesis of ascorbic acid is observed. Biosynthesis is brought about only by KCN concentration above $5 \times 10^{-3}M$, as was mentioned before. It appears possible that the biosynthesis of ascorbic acid from D-glucurono-γ-lactone is catalyzed by an enzyme system which is activated by cyanide, or that cyanide acts by blocking an alternative pathway of the metabolism of glucurone which might involve an iron-containing enzyme system, or that there is a combination of these actions.

Livers from other species were also examined for their ability to convert D-glucuronolactone under the influence of cyanide. The results show that there is a considerable difference in the ability of different species to effect this synthesis, as is determined by indophenol titration. Goat liver appears to be the most potent among the mammalian livers examined, while the livers of the guinea pig, the chick, and the pigeon appear to be incapable of effecting this conversion under the experimental conditions studied. An enzyme concentrate which brings about the synthesis of ascorbic acid from glucurone in the presence of cyanide has been prepared from goat liver (*5*).

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References and Notes

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2. G. H. Hogeboom, W. C. Schneider, G. E. Pallade, *ibid.* 172, 619 (1948).
3. J. H. Roe and C. A. Kuether, *ibid.* 147, 399 (1943).
4. This work was supported by financial assistance from the Indian Council of Medical Research.
5. A detailed report is in preparation.

10 July 1957

Composition of the Placental Septa as Shown by Nuclear Sexing

The composition and source of the septa placentae, the thin membrane situated between the cotyledons of the human placenta (Fig. 1), have long been argued. Opinions have until now been varied on whether the cells comprising this tissue were of fetal or maternal origin. Grosser (*1*) discussed the then prevalent opinions and his own observations and expressed the view that the septum is composed of maternal tissue with a minor element of trophoblastic cells. Recent studies (*2*), including those of Wislocki, in which histochemical and electron microscopic methods were used, seemed to show that the septum was composed mainly of trophoblastic cells, with perhaps a small maternal element at its basal end. Other recent works (*3*), however, have still maintained that the structure is a maternal one.

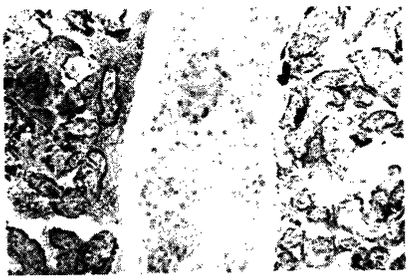

Fig. 1. Photomicrograph of a placental septum as it lies between adjacent cotyledons (×140).

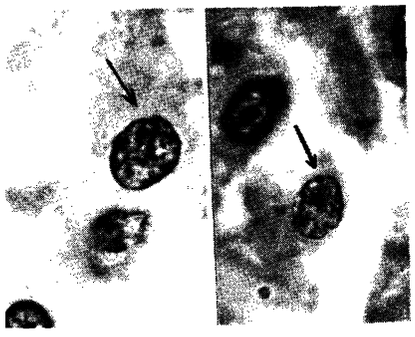

Fig. 2 (*a* and *b*). The same preparation as in Fig. 1, showing the cells of the septum under higher magnification (×1500). The arrows point to the sex chromatin bodies seen in the nuclei of these cells (Feulgen stain).

A study of the placental septa in the human being has been carried out by us, the method being based on the morphological sex difference now known to exist in the nuclei of intermitotic cells (*4, 5*). It has been shown that this method is applicable to a variety of mammalian, and to most human, tissues, including embryonic tissues (*6*). The material used included placentas from 14 male fetuses at term, sections being cut at 6 μ, stained by the Feulgen method (Fig. 2), and examined at a magnification of times 1200. A count was made on 100 cells in each case, by two independent observers.

In ten cases, the percentage of cells with sex chromatin in their nuclei was well above 50 percent, and in another four cases, the proportion was more than 35 percent. In no case was the count within the limits of a male picture (male fetal tissues have been shown to contain a mean 12 percent sex chromatin bodies, *4, 7*). Studies performed on various female tissues in the human being have shown that the percentage of nuclei