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 0.0125*M*. 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 0.01*M* cyanide. Thus, in a recovery experiment starting with pure L-ascorbic acid, it has been found that in the presence of 0.01*M* 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*).

I. B. CHATTERJEE, N. C. GHOSH
J. J. GHOSH, B. C. GUHA
Department of Applied Chemistry, University College of Science and Technology, Calcutta, India

References and Notes

1. M. ul Hassan and A. L. Lehninger, *J. Biol. Chem.* 223, 123 (1956).
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.

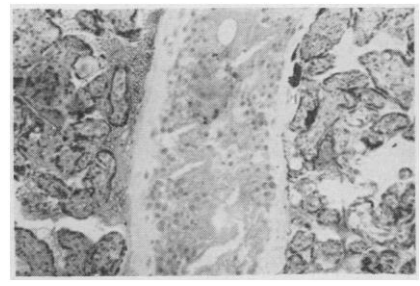

Fig. 1. Photomicrograph of a placental septum as it lies between adjacent cotyledons (×140).

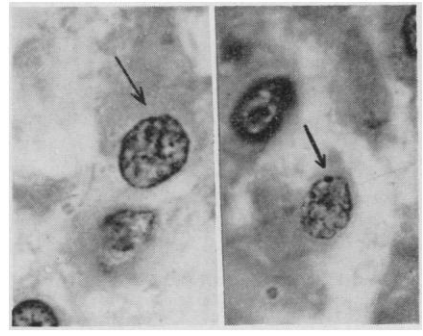

Fig. 2 (*a* and *b*). The same preparation as in Fig. 1, showing the cells of the septum under higher magnification (×1500). The arrows point to the sex chromatin bodies seen in the nuclei of these cells (Feulgen stain).

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

showing the presence of sex chromatin varies according to the type of tissue examined. Ranges vary from 52 to 85 percent, in skin biopsies, to as low as 20 to 64 percent for exfoliated cells. Embryonic tissue (amniotic fluid cells) has shown a range of 34 to 66 percent (*4, 7*). In view of these observations, we feel that the results obtained in the present investigation allow us to suggest that the placental septa are composed for the most part of cells which are of maternal origin (*8*).

ARYEH SADOVSKY
DAVID M. SERR
GERTRUDE KOHN
Rothschild-Hadassah University Hospital and *Hormone Research Laboratory, Hebrew University Hadassah Medical School, Jerusalem, Israel*

References and Notes

1. O. Grosser, *Frühentwicklung, Eihautbildung und Placentation des Menschen und der Säugetiere* (Bergmann, Munich, Germany, 1927), p. 373.
2. G. B. Wislocki, *Anat. Record* 109, 359 (1951); H. Stieve and I. von der Heide, *Anat. Anz.* 92, 1 (1941) and in *Gestation* (Josiah Macy, Jr., Foundation, 1956), p. 140–143; N. J. Eastman, *William's Obstetrics* (Appleton-Century-Crofts, New York, ed. 11, 1956).
3. J. P. Greenhill, *Obstetrics* (Saunders, Philadelphia, Pa., ed. 11, 1955), p. 38; A. E. Petchenko, *Obstetrics* (U.S.S.R. State Publication, Kiev, 1956), p. 59.
4. K. L. Moore, M. A. Graham, M. L. Barr, *Surg. Gynecol. Obstet.* 96, 641 (1953).
5. K. L. Moore and M. L. Barr, *Acta Anat.* 21, 197 (1954); J. L. Emery and M. McMillan, *J. Pathol. Bacteriol.* 68, 17 (1954); K. L. Moore and M. L. Barr, *Lancet* 269, 57 (1955); E. Marberger, R. A. Boccabella, W. O. Nelson, *Proc. Soc. Exptl. Biol. Med.* 89, 488 (1955) and *Lancet* 269, 654 (1955); B. Lennox, *Scot. Med. J.* 1, 97 (1956).
6. M. A. Graham, *Anat. Record* 4, 469 (1954); E. Marberger and W. O. Nelson, *Bruns' Beitr. klin. Chir.* 190, 103 (1955); D. M. Serr, L. Sachs, M. Danon, *Bull. Research Council Israel* 5B, 137 (1955).
7. W. F. Hunter and B. Lennox, *Lancet* 2, 633 (1954); K. L. Moore and M. L. Barr, *ibid.* 2, 57 (1955); K. L. Moore and M. L. Barr, *Brit. J. Cancer* 9, 246 (1955); A. D. Dixon and J. B. D. Tarr, *Brit. Med. J.* 2, 799 (1956); L. Sachs, D. M. Serr, M. Danon, *ibid.* 2, 795 (1956).
8. Details of an extended study of this problem are in preparation. We are indebted to B. Zondek for his interest during the course of this study and for facilities provided in his laboratory.

19 July 1957

Stimulant Effect of 2-Dimethylaminoethanol—Possible Precursor of Brain Acetylcholine

Pfeiffer and Jenney (*1*) have found that the tertiary amines arecoline, pilocarpine, and eserine will inhibit the conditioned avoidance response in rats protected peripherally with atropine methyl nitrate. Arecoline, when used in atropine methyl nitrate-protected schizophrenic patients, will produce a "lucid interval" similar to that of amobarbital or carbon dioxide (*1, 2*). These findings initiated a new research program (*3*) designed to find longer acting parasympathomimetic agents or precursors of acetylcholine which might affect the central nervous system. Two quaternary nitrogen compounds, acetylcholine and methacholine, probably owing to their slow transit across the blood-brain-barrier, are without effect on the conditioned avoidance response. Furthermore, Mayer and Bain (*4*) have used the tertiary and quaternary analogs of a convulsant, fluorescent acridone, to delineate the blood-brain-barrier, and Koelle and Steiner (*5*) find that the quaternary nitrogen analog of a phosphate ester of thio-choline inhibits the peripheral but not the cerebral cholinesterase, while the tertiary amine congenor inhibits both. These observations are not compatible with the concept that choline acts as an effective precursor of cerebral acetylcholine, because choline may be limited in its transport across the membranal barriers.

The lack of pharmacological potency of choline chloride in man is evidenced in the treatment of patients with liver disease, where as much as 10 g per day may be given without any discernible acetylcholinelike pharmacodynamic effect (*6*). Since choline is a methyl donor, one might expect the tertiary analog to be readily formed *in vivo*. However, choline as a methyl donor has been reported to go to dimethyl glycine by way of the intermediate, betaine (*7*), and, therefore, it may not provide a tertiary amine precursor for the synthesis of cerebral acetylcholine. For this reason, possible tertiary amine precursors of acetylcholine, including 2-dimethylaminoethanol (DMAE), have been studied extensively in mammals, including man, in these laboratories.

The pertinent publications on DMAE can be summarized briefly as follows: Krayer *et al.* (*8*) find that DMAE in a dose of 200 mg/800 ml volume counteracts the cardiac failure produced by pentobarbital sodium. Biochemical studies [Korey *et al.* (*9*)] indicate that DMAE is acetylated at the same rate as is choline by choline acetylase, and that acetyl-DMAE has less than 0.01 of the activity of acetylcholine on the frog rectus preparation. In chronic feeding experiments on growing chicks on a choline-deficient diet, Jukes and Oleson (*10*) report that DMAE will partially substitute for choline. In a *Neurospora* cholineless mutant, however, Jukes and Dornbush (*11*) show that DMAE substitutes completely for choline as a growth factor. Reid (*12*), working with choline-deficient guinea pigs, finds that DMAE will substitute completely for choline, whereas aminoethanol, N-methylaminoethanol, betaine, dimethyl glycine, and methionine are ineffective.

The following pharmacological observations have been made (*13*). The oral

Table 1. Effect of choline and DMAE on convulsant thresholds when 1.0 g per day is given orally for 2 weeks. Twelve mice were used in each group, and 0.5 percent pentylenetetrazol was infused intravenously at the rate of 0.05 ml/10 sec. $t = 2.392$ for difference between means of choline and DMAE. $p = < 0.05$.

Treatment	Lethal convulsion (ml/mouse)
Controls	0.457 ± 0.11
Choline	0.433 ± 0.09
DMAE	0.342 ± 0.11

LD_{50} of DMAE tartrate is 3.1 ± 0.16 g/kg, mouse, and 2.6 ± 0.1 g/kg, rat. With these large doses, animals show respiratory depression and frequently die of pulmonary edema. In pharmacodynamic studies on anesthetized dogs, DMAE tartrate has approximately 0.002 of the vasodepressor effect of acteylcholine, 0.1 of that of pilocarpine, and 0.5 of that of choline chloride. The muscarinic effect on the guinea pig uterus is approximately 0.1 and on the rabbit ileum, 0.025 of that of choline chloride (*13*). Rats and other species tolerate and grow normally on daily doses of 50 or 100 mg/kg for 6 months or longer. Higher doses (500 mg/kg a day) produce a small but statistically significant lowering of the hematocrit, hemoglobin, and eosinophil count. The animals grow normally, but occasional deaths occur from maximal seizures which appear in both rats and mice after 3 to 4 weeks of dosage. Mice treated chronically at 500 mg/kg a day are also more susceptible to audiogenic seizures (*13*). Daily oral dosage of DMAE significantly lowers the threshold for seizures from intravenously infused pentylenetetrazol (Tables 1 and 2). These data correlate with those obtained in curarized nonanesthetized cats, in which DMAE demonstrated some antagonism of the effects of pentobarbital on the electroencephalogram. The incidence of low-voltage fast activity in the electroencephalogram, similar to that obtained during arousal, was increased

Table 2. Effect of choline and DMAE on convulsant thresholds when they are fed to mice as a 0.03*M* solution in drinking water for 31 days. Ten mice were used in each group, and 0.5 percent pentylenetetrazol was infused intravenously at the rate of 0.05 ml/10 sec. $t = 5.3295$ for difference between means of choline and DMAE. $p = < 0.001$.

Treatment	Lethal convulsion (ml/mouse)
Controls	0.525 ± 0.09
Choline	0.525 ± 0.10
DMAE	0.250 ± 0.11