As the compound reduces the cerebral serotonin level, vincamin as well as reserpin and other alkaloids with similar structure is a new material found to effect the metabolism of serotonin. Our investigations showed however that this effect of vincamin is different from that of reserpin, being of a lesser degree and developing more slowly. The diminishing effect of vincamin on noradrenalin level is more intensive sooner, and more durable. The effects of vincamin on the noradrenalin and serotonin level are probably due to different modes of action.

Further investigations have to be carried out in order to elucidate the role the depletion of the serotonin level plays in the pharmacological and therapeutical effect of the compound.

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Passage of caffeine into human gonadal and fetal tissue

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CAFFEINE is known to be mutagenic to bacteria,[1] fungi,[2] and Drosophila.[3] No report of mutagenic activity in mammals has yet appeared. However, the basic similarity of the mechanisms for replication of the genetic material in all forms of life poses the possibility that caffeine may be mutagenic in man. Because of the widespread consumption of caffeinated beverages a major part of the human race is chronically exposed to this compound. Mutagenic effects of a drug could be of genetic significance at any time before or during a person's reproductive years. In fetal life, exposure might be especially hazardous at about 6–8 weeks, during the period of segregation and proliferation of the germ cells. Several aspects of this problem have been discussed elsewhere.[4]

A first step in assessing the potential genetic hazard of exposure to a mutagen is to ascertain whether or not the compound in question gains access to germinal tissue. Axelrod and Reichenthal[5] have already shown in the dog that caffeine distributes freely into intracellular water of all tissues examined. It was thought desirable, however, to look into the matter directly in humans, and specifically in ovary, testis, and fetus, none of which had been included in their investigation. Moreover, there were some grounds for supposing that during early gestation the placenta might not permit the drug to pass freely.[6] The experiments described here, although necessarily conducted with a small number of subjects, show quite clearly that caffeine equilibrates freely between plasma and tissue water in the case of human ovary and testis, and also between maternal plasma and the human 7–8 week fetus.

MATERIALS AND METHODS

Tissues. Human gonads were obtained as surgical specimens. Testes were from patients undergoing bilateral orchidectomy for cancer of the prostate. Ovaries were from patients having bilateral oophorectomy for cancer of the breast. All gonads appeared to be normal. Fetuses were from therapeutic abortions performed for psychiatric indications during the 7th–8th gestational week. One specimen was obtained as fragments from curettage, the other intact by hysterectomy.

Caffeine administration. In the case of the ovaries, a control ovary and a 10-ml sample of oxalated blood were first obtained. Then 1 g of caffeine sodium benzoate (574 mg caffeine) was given by intravenous infusion over a period of 5 min. After a further 15–20 min for equilibration, the second ovary was removed and another blood sample drawn. The tissue and plasma controls proved to be unnecessary inasmuch as they contained no apparent caffeine. Zero-blanks were also found with one control testis and with control samples of fetal liver. Therefore, in the experiments with testes and on e

of the two fetuses, the procedure for administering caffeine was simplified. The same dose of caffeine sodium benzoate was given intramuscularly at least 30 min prior to the operative procedure, and a single blood sample was drawn simultaneously with removal of the tissue. All tissue samples were refrigerated immediately and stored at −20°C until analysed.

Caffeine analysis. The method of Axelrod and Reichenthal[5] was used, with the following modifications. Tissues were minced and extracted three times in M/10 phosphate buffer, pH 7·4, at 90°C. The combined supernatant fluids were then extracted into benzene in the presence of salt, as in the original method. The final 5N HCl solution was found to contain traces of benzene, which contributed an interfering peak in the ultraviolet spectrum at 260 mμ; this was removed readily by heating. The 5N HCl solutions, including appropriate blanks and standards, were read on the Beckman DK recording spectrophotometer. At this acidity, the absorption peak of caffeine is at 266 mμ (Fig 1).

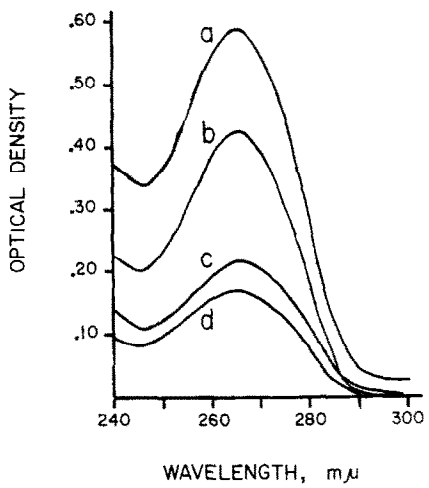

FIG. 1. Absorption spectra of caffeine extracted from tissues: (a) testis; (b) ovary; (c) caffeine standard; (d) fetus.

Over-all recovery of caffeine added to tissue controls was 70 per cent; experimental data were corrected for this loss, largely due to poor extraction in the initial step. Plasma was analysed by the original method without modification; recovery was 86 per cent or better. The analytical procedure is specific for caffeine; all of its metabolites are removed during the solvent extractions.

RESULTS AND DISCUSSION

The data are presented in Tables 1, 2 and 3. It is obvious that the caffeine concentrations, although not identical, were essentially the same in ovary and testis water as in plasma. In one fetus, the concentration of caffeine was the same as in maternal plasma. In the other fetus, caffeine was also present,

TABLE 1. CAFFEINE CONTENT OF OVARY

Patient	Age	Equilibration time* (min)	Caffeine concentration (μg/ml)	
			Ovary†	Plasma
K	47	20	26·5	30·0
N	43	15	23·0	21·5

* After intravenous administration.

† The caffeine concentration is expressed in μg per ml of tissue water, assumed to be 70 per cent of wet-weight.

but its concentration appeared to be somewhat lower than in maternal plasma; however, the accuracy of the assay at such low concentrations leaves much to be desired.

These experiments show that caffeine has access to human adult and fetal gonads and that it achieves, in these tissues, concentrations substantially the same as in plasma. If a man or woman drinks coffee or strong tea several times daily, his (or her) germ cells are bathed in a caffeine solution

TABLE 2. CAFFEINE CONTENT OF TESTIS

Patient	Age	Equilibration time* (min)	Caffeine concentration (μg/ml)		
			Right testis†	Left testis†	Plasma
R	60	75	10·9	10·3	9·8
S	86	120	9·0	8·9	9·8

* After intramuscular administration. The time given includes the absorption time.

† The seminiferous tubule mass was dissected free of all accessory tissues. The caffeine concentration is expressed in μg per ml of tissue water, assumed to be 70 per cent of wet-weight.

TABLE 3. CAFFEINE CONTENT OF FETUS

Patient	Weight of fetus (g)	Equilibration time (min)	Caffeine concentration (μg/ml)	
			Fetus*	Maternal plasma
H	1·72	50 (intramuscular injection)	1·9	3·5
L	2·73	40 (intravenous injection)	9·9	10·2

* The gestational age of both fetuses was 7–8 weeks. The fetus from patient H was obtained as fragments from curettage, that from patient L intact by hysterectomy. Fetal caffeine concentration is expressed in μg per ml of tissue water, assumed to be 70 per cent of wet-weight.

of fluctuating concentration, estimated as equivalent to a continuous exposure at about 1 μg/ml.[4] In a pregnant woman the germ cells of the fetal gonads would be exposed to a similar concentration. Whether, and under what conditions, such chronic exposure of the human germ line to caffeine may constitute a genetic hazard is at present entirely unknown.

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