Biochemical Pharmacology, Vol. 29, pp. 1663–1668.

0006-2952/80/0615-1663 $02.00/0

CHARACTERISTICS OF DRUGS THAT PENETRATE THE PREIMPLANTATION BLASTOCYST

AMNUAY THITHAPANDHA
Department of Pharmacology, Faculty of Science, Mahidol University, Bangkok, Thailand

(*Received* 13 *December* 1979; *accepted* 24 *January* 1980)

Abstract—Experiments were performed to evaluate the role of physicochemical properties of drugs and chemicals in determining their passage into the preimplantation blastocysts of 6-day pregnant rabbits. It was found that compounds with molecular weight less than ouabain (mol. wt 585) readily traversed the blastocyst, whereas the blastocyst was relatively impermeable to those with molecular weight greater than 5000. However, for compounds with small molecular weights (100–300), the blastocyst/plasma radioactivity ratios varied considerably (0.001–1.24), suggesting that factors other than molecular weight played a role in determining their passage. Further experiments revealed that there was no correlation between blastocyst/plasma radioactivity ratio and degree of protein binding of the drugs. It was concluded that the degree of ionization and lipid solubility of the compounds are important factors in determining their rates of penetration into the preimplantation blastocysts. These findings prove that a variety of foreign compounds can enter the blastocyst during the preimplantation stages of gestation and their rates of penetration are governed by the possible interactions among their physicochemical properties. The toxicological implications of these findings were also discussed.

Recent studies have shown that a variety of drugs and chemicals pass into the preimplantation blastocyst and the developing fetus quite readily [1–3]. Following the *in vivo* administration of caffeine, nicotine, DDT, barbital, thiopental and isoniazid to pregnant rabbits, these substances can be identified in the preimplantation blastocyst [1, 3]. Incubation of the rabbit blastocyst with drugs *in vitro* results in drug uptake; the rate of uptake correlates closely with the lipid solubility and degree of ionization of each compound [3, 4]. However, little is known about the possible factors which may influence the transfer of compounds from the general circulation into either the uterine secretion or the preimplantation blastocyst.

This report represents an evaluation of the factors which are important in the transfer of drugs into the preimplantation blastocyst after their oral or parenteral administration. Salicylate, antipyrine, nicotine, DDT, caffeine, hexamethonium, inulin, isoniazid, barbiturates, ouabain, tetracycline, diphenylhydantoin, phenylbutazone and several other compounds and antimicrobials were selected for these studies. This is because they are compounds with a broad spectrum of physicochemical characteristics, and some of them are among the most commonly used in our society. These compounds were used to evaluate the relative importance of molecular weight, lipid solubility, degree of ionization and protein binding as factors in their transfer from the blood into the preimplantation blastocyst.

MATERIALS AND METHODS

Chemicals. The following radioactive compounds were purchased from the manufacturers indicated: C2-^{14}C-barbital (1.10 mCi/mmole) and 2-^{14}C-thiopental (5.19 mCi/mmole) from Tracerlab (Waltham, MA); carboxy-^{14}C-isonicotine acid hydrazide or isoniazid (9.8 mCi/mmole) and G-^{3}H-nicotine (210 mCi/mmole) from Nuclear Chicago Corporation (Des Plaines, IL); carboxy-^{14}C-salicylic acid (1.5 mCi/mmole), *N*-methyl-^{14}C-antipyrine (0.5 mCi/mmole), 1-methyl-^{14}C-caffeine (5.0 mCi/mmole), methyl-^{14}C-hexamethonium (1.55 mCi/mmole); 1-^{14}C-tetraethylammonium (1.15 mCi/mmole), ^{3}H-ouabain (1.46 mC/g),1,1-bis-*p*-chlorophenyl-^{14}C-2,2,2-trichloroethane (*p,p'*-DDT;2.73 mCi/mmole),4-^{14}C-5, 5-diphenylhydantoin (55 mCi/mmole), carboxy-^{14}C-inulin (3.96 mCi/g) and carboxy-^{14}C-dextran (mol. wt. 16,000–19,000; 2.49 mCi/g) from New England Corporation (Boston, MA); and ^{3}H-dihydrostreptomycin, sequisulfate (2.1 mCi/mg); 7-^{3}H-tetracycline (3 mCi/mg) and ^{14}C-chloramphenicol (70 mCi/mg) from Radiochemical Center (Amersham, U.K.). All other nonradioactive materials were of analytical grade and obtained commercially. The purity of these compounds was checked by thin-layer chromatography.

Chemical and physical measurements. The analytical methods of Goldbaum and Smith [5], as modified by Schanker *et al.* [6], were used for the estimation of the barbiturates (barbital, secobarbital and thiopental). Phenylbutazone was estimated by the method of Burns *et al.* [7].

Chloroform/Ringer phosphate partition coefficients ($K_{chloroform}$) of the undissociated forms of the drugs were determined by the method of Hogben *et al.* [8], using unlabeled compounds.

Animal experiments. Bucks and does weighing 3–4 kg were purchased from the local market. They were kept in our animal house for at least three or four days before they were mated with each other.

The time of mating was considered hour zero of pregnancy. All animals were allowed free access to food and water. Pregnancy was assumed to have begun if the female revealed behavioral after-reactions to coitus, as described by Sawyer and Kawakami [9]. The pregnant animals were then treated at 140–144 hr after pregnancy.

Each radioactive compound at the dose indicated in the tables was administered either by gavage in approximately 5–6 ml of water or by intravenous administration in saline or ethanol in a volume of less than 2.0 ml. Each ^{3}H or ^{14}C compound and their metabolites were identified by inverse radioisotope dilution technique after solvent extraction and chromatographic separation, as described in detail by Sieber and Fabro [3]. In the present study, the following compounds and metabolites were identified in the plasma and blastocyst: barbital, isoniazid and its two metabolites, salicylate, thiopental, caffeine and its four metabolites, and nicotine and its two best known metabolites (cotinine and demethylcotinine).

The treated animals were stunned by a blow on the head at appropriate times and killed by exsanguination. Blood samples (10–20 ml) were then collected from the jugular vein into heparin-treated test tubes, and the plasma was obtained by centrifuging the blood at 800 *g* for 10 min. The uterus was rapidly exposed and opened, and the free blastocysts, weighing 20–60 mg each, were removed.

Measurement of radioactivity was carried out by dispersing blastocysts (3–6) or plasma (0.1–0.5 ml) in sufficient liquid scintillation fluid in glass counting vials to give a final volume of approximately 20 ml. The scintillation fluid consisted of a dioxane–ethylene–methanol mixture (44 : 1 : 5 v/v) containing naphthalene (6%), 2, 5-diphenyloxazole (0.4%), 1,4-bis (5-phenyloxazole-2-yl) benzene (0.02%) and thixotrophic gel powder (Cab-O-Sil, 5%). Radioactivity was measured in a Packard liquid scintillation spectrometer, model 3003, after the samples had been left in the counter for 16–24 hr. Counting efficiency (approximately 70 per cent) was determined by the external standard method (Wang and Willis, 1965). pH values were measured electrometrically by using a Beckman expandometric SS-2 pH meter.

Experiments in vitro. Twenty blastocysts obtained from 6-day pregnant rabbits were pooled and incubated in 10 ml of Ringer–phosphate buffer, pH 7.2, containing the appropriate unlabeled barbiturate (barbital, secobarbital and thiopental) at a final concentration of 5×10^{-3}M. The Ringer–phosphate, pH 7.2, was prepared according to Davson and Eggleton [10]. Incubations were carried out at 37° in air atmosphere with an Aminco constant temperature water bath. At intervals of 2, 5, 8, 10, 20, 30, 45, 60, 90 and 120 min after the incubation had begun, two blastocysts were removed from the medium, blotted dry, weighed and homogenized for the determination of barbiturate. Duplicate samples (0.1–0.2 ml) of the incubation medium were also removed at the same time, and the barbiturate concentration determined.

The penetration of the barbiturates into the rabbit blastocyst was expressed on the basis of the relative amount of drug concentration in the blastocyst as compared to that in the incubation medium. Equilibrium is attained when an increase in the time of incubation does not result in a concomitant increase in the ratio barbiturate concentration in blastocyst/barbiturate concentration in medium. Half-equilibrium time ($T_{1/2}$) has been used as a measure of the rate of penetration of the compounds into the blastocyst. $T_{1/2}$ is defined as the time (in minutes) which is required for a compound, incubated under the conditions described above, to reach a concentration in the blastocyst equal to one-half of that at equilibrium. $T_{1/2}$ was obtained, as shown in Table 4, by calculating a regression line of the plot with the reciprocal values of the ratio barbiturate concentration in blastocyst/barbiturate concentration in medium versus the reciprocals of the corresponding time of incubation (in minutes). By this procedure, a straight line was obtained which describes the rate of penetration into the blastocyst of each of the compounds studied. The intercept of this line on the abscissa corresponds to the reciprocal value of $T_{1/2}$.

RESULTS

In all experiments reported here, the blastocyst/plasma radioactivity ratio of rabbits treated with a labeled compound was used as an index of the degree of its penetration into the preimplantation blastocyst. This ratio was determined after the level of radioactivity in the plasma had reached a steady state which was considered to be attained when the decline in the plasma level of radioactivity, following the initial rapid fall, was linear when plotted semilogarithmically [11, 12]. Depending on the nature of a particular compound and route of administration, the time required to reach the steady state was found experimentally to range from 15 min to 4 hr. For this reason, the distribution of radioactivity between blastocyst and plasma was determined 1 or 6 hr after dosing with the labeled chemicals studied.

Table 1 shows the distribution of radioactivity of a variety of drugs and chemicals into the preimplantation blastocyst of 6-day pregnant rabbits treated with such labeled compounds. It is evident that acids and bases selected in the present study entered the preimplantation blastocyst quite readily, although not to the same degree. It should be noted that though the blastocyst contained less radioactivity of 2-^{14}C-thiopental than the plasma at 1 hr, by 5 hr the radioactivity in the blastocyst was approximately equal to that of the maternal plasma (the blastocyst/plasma ratio being 0.98 ± 0.12, 3 rabbits). The distribution pattern of DDT in the rabbit blastocyst was similar to that of thiopental, but the ratio of ^{14}C in blastocyst/^{14}C in plasma never exceeded 0.24 and was highest at 1 hr after treatment (0.24); at 6 and 24 hr this ratio was 0.12 and 0.13, respectively. Of particular interest was the finding that the blastocyst/plasma radioactivity ratio of nicotine exceeded 1; it was 2.06 ± 0.20. However, ionized compounds such as hexamethonium and tetraethylammonium hardly entered the preimplantation blastocyst. The distribution of ouabain (mol. wt 585) was somewhat higher than would be expected on the basis of its lipid solubility and molecular weight. These three

Table 1. Concentration ratios of radioactivity in preimplantation blastocyst and plasma of 6-day pregnant rabbits treated with ^{14}C- or ^{3}H-labeled compounds

Compound	pK_a*	$K_{chloroform}$ †	Route	Dose (mg/kg)	Dose (μCi/kg)	Length of experiment (hr)	Blastocyst‡ / Plasma
Acid							
2-^{14}C-Barbital	7.8	0.7	P.O.	100	10	6	1.56 ± 0.32
Carbonyl-^{14}C-isoniazid	3.8	0.04	P.O.	13	5	6	1.04 ± 0.10
Carboxy-^{14}C-salicylic acid	3.0	2.9	P.O.	17	5	4	0.40 ± 0.02
2-^{14}C-Thiopental	7.6	100	I.V.	20	10	1	0.24 ± 0.02
Base							
N-Methyl-^{14}C-antipyrine	1.4	21.2	I.V.	100	20	1	0.78 ± 0.04
1-Methyl-^{14}C-caffeine	0.8	23	P.O.	3.5	5	6	0.82 ± 0.10
G-^{3}H-Nicotine	—	—	I.V.	0.05	60	1	2.06 ± 0.20
Others							
Methyl-^{14}C-hexamethonium	(cation)	—	I.V.	0.60	5	1	N.S.
1-^{14}C-Tetraethylammonium	(cation)	—	I.V.	0.92	7.2	1	N.S.
^{3}H-Ouabain	—	0.004	I.V.	0.60	0.87	1	0.04 ± 0.002
Phenyl-^{14}C-DDT	—	1000	I.V.	0.4	3.3	1	0.24 ± 0.01

* From Sieber and Fabro [3].

† Partition coefficients were determined after distributing the drugs between chloroform and phosphate buffer, pH of which was such that the drug was largely in the unionized form [8].

‡ Expressed as d.p.m./g/d.p.m./ml. Means ± S.E.M. of three to four experiments in duplicate. N.S. = not significant; the blastocyst–plasma ratio was 0.001 or less.

compounds did not approach equilibrium with plasma during the 3 or 5 hr period of study.

As shown in Table 2, there was an inverse relationship between molecular weights of compounds and their blastocyst/plasma radioactivity ratios. This correlation was statistically significant ($P < 0.001$). It is also apparent that compounds having molecular weights greater than 600 (ouabain = 585) did not seem to be able to penetrate the preimplantation blastocyst. However, an examination of the results shown in Table 3 reveals that for compounds with small molecular weights (100–300) the blastocyst/plasma ratios varied considerably (0.001–1.24), suggesting that factors other than molecular weight played a role in determining this ratio. It can also be seen that the rates of distribution of xenobiotic agents into the blastocyst correlate roughly in a general way with their degree of ionization (Table 3). Antipyrine, caffeine and isoniazid, present almost exclusively in the unionized forms at pH 7.4, penetrated the blastocyst rapidly, whereas thiopental, which is 50 per cent ionized at physiologic pH, entered the blastocyst less rapidly, and it took approximately 5 hr before the ratio of ^{14}C in blastocyst/^{14}C in plasma would approach a value of 1.0. Salicylic acid, which is more than 99 per cent ionized at pH 7.4, entered the blastocyst more than would be expected. Nevertheless, its blastocyst/plasma radioactivity ratio did not exceed 0.46 during any period of the experiment (5 hr). Hexamethonium, a cation, hardly entered the blastocyst (Table 3).

An indication that lipid solubility is the most important factor in determining the rate of passage of xenobiotics into the preimplantation blastocyst is

Table 2. Passage of some compounds into the preimplantation blastocyst of 6-day pregnant rabbits 1 hr after their intravenous administration

Compound	Mol. wt	Dose (mg/kg)	Dose (μCi/kg)	Blastocyst* / Plasma
2-^{14}C-Barbital	184	100	10.0	1.48 ± 0.12
Phenyl-^{14}C-DDT	355	0.7	5.0	0.26 ± 0.10
^{3}H-Ouabain	585	0.6	0.87	0.04 ± 0.002
Carboxy-^{14}C-inulin	5500	2.7	5.0	0.004 ± 0.001
Carboxy-^{14}C-dextran	17,700	1.1	5.0	< 0.001

* Values expressed in (d.p.m./g)/(d.p.m./ml); means ± S.E.M. of three experiments in duplicate. With the exception of ^{3}H-ouabain, these compounds were not metabolized by one hour after their injection, since all the radioactivity in plasma for each chemical was identified as the unchanged compound. There was an inverse relationship between the molecular weights of the compounds and blastocyst/plasma radioactivity ratios ($P < 0.001$).

Table 3. Penetration of radioactive compounds into preimplantation blastocyst of 6-day pregnant rabbits 1 hr after their intravenous administration

Compound	Dose (mg/kg; μCi/kg)	pK_a*	Mol. wt	% ionized at pH 7.4	Blastocyst† / Plasma
N-Methyl-^{14}C-antipyrine	4.5; 5	1.4	188	< 0.01	0.90 ± 0.18
1-Methyl-^{14}C-caffeine	3.5; 5	0.8	194	< 0.01	1.20 ± 0.12
Carboxy-^{14}C-isoniazid	13; 5	3.8	137	0.03	1.24 ± 0.10
2-^{14}C-thiopental	20; 10	7.4	242	50	0.24 ± 0.02
Carboxy-^{14}C-salicylic acid	17.0; 5	3.0	138	> 99	0.46 ± 0.02
Methyl-^{14}C-hexamethonium	0.6; 5	(cation)	273	> 99	< 0.001

* Values obtained from Sieber and Fabro [3].

† Expressed as d.p.m./g/d.p.m./ml; means ± S.E.M. of four experiments in duplicate. The degree of this transfer into the blastocyst was statistically correlated to their degree of ionization ($P < 0.05$).

Table 4. Comparison between blastocyst uptake of barbiturates and lipid–water partition coefficient of the undissociated form of the barbiturate

Barbiturate	pK_a*	$K_{heptane}$†	K_{MeCl}*	$T_{\frac{1}{2}}$ (min)‡	% Ionized at pH 7.2
Barbital	7.8	0.001	1	9.5	20
Secobarbital	7.9	0.10	52	4.6	17
Thiopental	7.4	3.30	580	1.5	39

* Values obtained from M. T. Bush [23].

† Values from L. S. Schanker [24].

‡ Half-equilibrium time—the time (min) which is necessary for a compound, incubated under the conditions described in Materials and Methods, to reach a concentration in the blastocyst equal to half of that at equilibrium.

Table 5. Effect of protein binding on the penetration of some radioactive compounds 1 hr after their intravenous administration into preimplantation blastocyst of 6-day pregnant rabbits

Compound	Dose (mg/kg; μCi/kg)	% Plasma protein binding	Blastocyst* / Plasma	Ref.
^{3}H-Dihydrostreptomycin	20 5	14	0.44 ± 0.10	[25]
7-^{3}H-Tetracycline	20 5	28	0.65 ± 0.08	[25]
^{14}C-Chloramphenicol	50 10	33	0.26 ± 0.11	[25]
Carboxy-^{14}C-salicylic acid	17 5	70	0.46 ± 0.02	[26]
2-^{14}C-Thiopental	20 10	75	0.24 ± 0.02	[26]
4-^{14}C-5, 5-diphenylhydantoin	4 5	90	0.40 ± 0.05	[27]
Phenylbutazone	20 –	98	0.12 ± 0.01	[7]

* Expressed as (d.p.m./g)/(d.p.m./ml); phenylbutazone, determined by the method of Burns *et al.* [7], was in (μg/g)/(μg/ml). Means ± S.E.M. of three to four experiments in duplicate. There was no correlation between the extent of plasma protein binding and the blastocyst/plasma ratio ($P > 0.05$).

provided by a study of three barbiturates of similar pK_a value (Table 4). Thiopental, with the highest lipid solubility or partition coefficient, was rapidly taken up by the blastocyst and thus had an equilibrium half-time ($T_{1/2}$) of less than 2 min. Barbital, which is less lipid soluble than thiopental, had a $T_{1/2}$ value of 9.5 min. Thus, the pK_a value of a drug gives only a rough indication of what the penetration rate will be, because it tells only what fraction of the drug molecules is present in the lipid-soluble unionized form. Some compounds such as the salicylates, which are completely ionized at pH 7.4, were found to cross the placental membranes and blastocyst barrier quite readily [13], perhaps because their ionized moieties are lipid soluble.

Table 5 demonstrates that the fractional binding to plasma proteins of several well-known drugs had little influence on the passage of radioactivity into the preimplantation blastocyst; the correlation between the extent or degree of binding and the blastocyst/plasma radioactivity ratio was not significant ($P > 0.05$).

A further attempt was made to quantitate some of the parent compounds and their metabolites, both in the plasma and in the preimplantation blastocyst. This is shown in Table 6. It can be seen that, following the oral administration of barbital (100 mg/kg; 8 μCi/kg), most of the radioactivity found in the blastocyst as well as in the plasma was associated with the unchanged drug. Table 6 also shows that

Table 6. Radioactive compounds identified in maternal plasma and preimplantation blastocyst of 6-day pregnant rabbits receiving some ^{14}C- or ^{3}H-labeled compounds

Compound administered*	Compound identified	Concentration in: Plasma (μg/ml)†	Blastocyst (μg/g)‡
2-^{14}C-Barbital	Barbital	88 (92.4)§	117 (92.3)§
Carbonyl-^{14}C-isoniazid	Isoniazid	0.96 (61.2)	0.97 (48.6)
	Acetylisoniazid	0.20 (13.4)	0.39 (19.5)
	Isonicotinic acid	0.33 (21.0)	0.35 (17.2)
Carboxy-^{14}C-salicylic acid	Salicylate	56 (30.4)	44 (60.4)
2-^{14}C-Thiopental	Thiopental	0.30 (39.0)	0.28 (46.6)
1-Methyl-^{14}C-Caffeine	Caffeine	2.35 (56.6)	2.49 (60.8)
	1, 2-Dimethylxanthine plus 1, 7-Dimethylxanthine	0.90 (23.0)	0.27 (8.0)
	1-Methylxanthine	0.05 (1.2)	(< 0.5)
	1, 3-Dimethyluric acid	0.25 (6.0)	0.03 (0.8)
G-^{3}H-nicotine	Nicotine	0.02 (24.2)	0.05 (42.0)
	Cotinine	0.03 (45.5)	0.03 (22.0)
	Demethylcotinine	0.005 (6.0)	0.002 (1.6)

* 2-^{14}C-barbital (100 mg/kg; 8 μCi/kg), carbonyl-^{14}C-isoniazid (13 mg/kg; 5 μCi/kg), carboxyl-^{14}C salicylate (17 mg/kg; 5 μCi/kg) and 1-methyl-^{14}C-caffeine (3.5 mg/kg; 5 μCi/kg) were given by stomach tube. Concentrations in plasma and blastocyst were examined 4 hr later for salicylate and 6 hr later for the rest. 2-^{14}C-thiopental (7 mg/kg; 7 μCi/kg) and G-^{3}H-nicotine (50 μg/kg; 60 μCi/kg) were given intravenously, and tissues were examined 5 and 1 hr later, respectively.

† Mean of the values found in two rabbits.

‡ Mean obtained from pool of six blastocysts.

§ Percentage of radioactivity in either plasma or blastocyst, respectively.

the blastocyst could be penetrated not only by the parent drugs but also by their metabolites. Isoniazid and two of its metabolites, acetylisoniazid and isonicotinic acid, were all detected in the blastocyst and their levels were comparable to those in the plasma. Caffeine and its metabolites, especially the dimethyl derivatives, were also found in the blastocyst, though the levels of its metabolites were much less than those in the plasma. For nicotine, the parent drug was found in much higher concentration than in the plasma, suggesting an active transport process or preferential higher protein binding by the blastocyst's proteins. The blastocyst was also well penetrated by the two metabolites, cotinine and demethylcotinine, since their levels in the blastocyst and in the plasma were approximately equal. Unchanged salicylic acid and unchanged thiopental represented about 60 and 42 per cent in the blastocyst, respectively.

DISCUSSION

The blastocyst/plasma ratio was utilized to assess the role of molecular weight, degree of ionization, protein binding and lipid solubility in the transfer of foreign compounds into the preimplantation blastocyst. Generally, for compounds ranging in molecular weight from less than 200 to about 17,700, the blastocyst/plasma ratio decreased with increasing molecular weight, inulin (mol. wt 5500) and dextran (mol. wt 17,700) being virtually excluded from the blastocyst. In fact, the radioactivity ratios of these compounds could be correlated with their molecular weight (Table 2). Furthermore, closer examination revealed that compounds having molecular weight greater than ouabain (585) would not have easy access into the preimplantation blastocyst.

For antipyrine, caffeine, isoniazid, thiopental, salicylic acid, hexamaethonium and DDT (compounds with molecular weight less than 500), the average blastocyst/plasma radioactivity ratios ranged from less than 0.001 to 1.24 ± 0.10 (Tables 2 and 3), indicating that the molecular weight of compounds is not the only factor which influences their transfer into the blastocyst. Thus, the degree of ionization of a compound appears to be important in determining its transfer into the preimplantation blastocyst (Table 3). This was also found to be the case with the extent or degree of lipid solubility (Table 4). On the other hand, no statistically significant correlation could be found between the blastocyst/plasma radioactivity ratio and the degree of binding to plasma proteins (Table 5).

It must be emphasized here that the relationships between blastocyst/plasma radioactivity ratios and any single property of the compounds such as molecular weight, degree of ionization, lipid solubility or protein binding may be obscured by the possible interactions among such properties. For example, caffeine and antipyrine have blastocyst/plasma radioactivity ratios close to one; although the chloroform/Ringer phosphate partition coefficient for caffeine is equal to that of antipyrine (Table 1), caffeine is more extensively bound to plasma proteins [8, 14].

These results, however, are not unexpected, since some of these factors have been shown to be important in the transfer of drugs into other exocrine secretions. Thus, molecular weight is important in the transfer of foreign chemicals into saliva [15] and uterine secretion [3, 4] and the degree of ionization

has been shown to influence the transfer of compounds into sweat [16], saliva [17] and milk [18].

The fact that these drugs and their metabolites do penetrate the preimplantation blastocyst (Table 6) may have toxicological implications. For example, it is well known that women who smoke are subject to increased risk of infertility [19] and have a higher abortion rate [20]. Becker and King [21] found that nicotine given to pregnant rats decreased the body weight of offspring, although it was not teratogenic. Al-Hachim and Fink [22] reported that the administration of DDT to mice late in pregnancy slowed the maturation of the nervous system of the newborn as measured by delays in the acquisition of the condition avoidance response. On the other hand, isoniazid given to tuberculous patients daily for more than 6 months has not been reported to produce any congenital abnormalities in the newborns. Thus, it has not been at all clear whether the presence of a drug in the blastocyst has a definite influence on the ultimate development of the fetus, and this is an area which awaits future toxicological evaluation.

Acknowledgements—The author is indebted to the professional staff of the Animal Care Committee, Faculty of Science, Mahidol University, for cooperation in securing the rabbits used in these studies. The work was supported by a grant-in-aid from the World Health Organization (Small Contract Programme H9/181/52C).

REFERENCES

1. S. Fabro and S. M. Sieber, *Nature, Lond.* **223**, 410 (1969).
2. C. Lutwak-Mann, M. F. Hay and D. A. T. New, *J. Reprod. Fert.* **18**, 235 (1969).
3. S. M. Siber and S. Fabro, *J. Pharmac. exp. Ther.* **176**, 65 (1971).
4. S. Fabro, in *Fetal Pharmacology*, (Ed. L. Boreus), p. 443. Raven Press, New York (1973).
5. L. R. Goldbaum and P. K. Smith, *J. Pharmac. exp. Ther.* **111**, 197 (1954).
6. L. S. Schanker, P. A. Shore, B. B. Brodie and C. A. M. Hogben, *J. Pharmac. exp. Ther.* **120**, 528 (1957).
7. J. J. Burns, R. K. Roscoe, T. Chenkin, A. Goldman, A. Schulert and B. B. Brodie, *J. Pharmac. exp. Ther.* **109**, 346 (1953).
8. C. A. M. Hogben, D. J. Tocco, B. B. Brodie and L. S. Schanker, *J. Pharmac. exp. Ther.* **125**, 275 (1959).
9. C. H. Sawyer and M. Kawakami, *Endocrinology* **65**, 622 (1959).
10. H. Davson and M. G. Eggleton, *Starling's Human Physiology*, 13th Edn, p. 39. Lea & Febiger, Philadelphia (1962).
11. B. B. Brodie and J. Axelrod, *J. Pharmac. exp. Ther.* **98**, 79 (1950).
12. A. Goldstein, L. Aronow and S. M. Kalman, *Principles of Drug Action*, p. 130. Harper & Row, New York (1968).
13. Y. Oh and B. L. Mirkin, *Fedn. Proc.* **30**, 2034 (1971).
14. B. B. Brodie, in *Absorption and Distribution of Drugs*, (Eds. T. B. Binns and C. Dodds), p. 16. Williams & Wilkins, Baltimore (1964).
15. K. Martin and A. S. V. Burgen, *J. gen. Physiol.* **46**, 225 (1962).
16. S. W. Brusilow and E. H. Gordes, *Am. J. Dis. Child.* **112**, 328 (1966).
17. S. A. Killman and J. H. Thaysen, *Scand. J. clin. Lab. Invest.* **7**, 86 (1955).
18. G. E. Miller, N. C. Banerjee and C. M. Stowe, *J. Pharmac. exp. Ther.* **157**, 245 (1967).
19. G. K. Tokuhata, *Archs environ. Hlth* **17**, 353 (1968).
20. J. M. O'Lane, *Obstet. Gynec.* **22**, 181 (1963).
21. R. F. Becker and J. E. King, *Am. J. Obstet. Gynec.* **95**, 515 (1966).
22. G. M. Al-Hachim and G. B. Fink, *Psychopharmacologia* **12**, 424 (1968).
23. M. T. Bush, in *Physiological Pharmacology* (Eds. W. S. Root and F. G. Hoffman), Vol. 1, Part A, p. 185. Academic Press, New York (1967).
24. L. S. Schanker, *J. Pharmac. exp. Ther.* **120**, 528 (1957).
25. G. Ziv and F. G. Sulman, *Antimicrob. Ag. Chemother.* **2**, 206 (1972).
26. B. B. Brodie, H. Kurz and L. S. Schanker, *J. Pharmac. exp. Ther.* **130**, 20 (1960).
27. P. K. M. Lunde, A. Rane and S. J. Yaffe, *Clin. Pharmac. Ther.* **11**, 846 (1970).