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Note: This review covers the literature through July 1968. Since that time many significant advancements have been made in the field of automation. Most notable of these are laboratory computer technology and automated sample injection in chromatography systems.

RESEARCH ARTICLES

Comparison and Analysis of the Teratogenic Effects of Serotonin, Angiotensin-II, and Bradykinin in Mice

ROBERT S. THOMPSON* and RONALD F. GAUTIERI

Abstract □ The incidence of anomalies in untreated and saline-treated controls (both s.c. and i.v.) was 1.5 and 0.26 percent, respectively. Subcutaneous administration of serotonin at 1 mg./mouse and 10 mg./kg. produced respective incidences of 24.4 and 27.0 percent, while i.v. administration caused total litter resorption. Angiotensin, 10 mg./kg., *via* both routes proved to be not significantly teratogenic. Bradykinin, 25 mcg./mouse s.c. produced 0.87 percent malformations, while the i.v. route caused 12.7 percent. The two most teratogenic agents, serotonin and bradykinin, both markedly decreased and increased, respectively, the transfer of radio-^{24}sodium from the maternal blood through the placental barrier to the fetal side of the placenta; angiotensin only slightly decreased this transfer. In addition, the three autocoids were noted to decrease the transfer of isotope from the placenta to the fetus, indicating that vasoconstriction of the fetal placental vessels may have occurred.

Keyphrases □ Teratogenic effects, mice—autocoids □ Serotonin—teratogenic effects, mice □ Angiotensin-II—teratogenic effects, mice □ Bradykinin—teratogenic effects, mice □ ^{24}Sodium, placental transport—autocoids effect □ Placental, uterine vessels—serotonin, angiotensin, bradykinin effect

Serotonin, angiotensin, and bradykinin, all autocoids, have several physiological actions in common. They are potent placental vasoconstrictors (1–4), affect permeability of blood vessels, affect blood pressure, produce some effect on kidney function, and have all been implicated in the production of various pathological states such as carcinoid syndrome, toxemia of burns, pregnancy, and allergy (5–9). Furthermore, they all stimulate various smooth muscle preparations and cause uterine contracture.

It becomes apparent that the administration, endogenous release, or even blockage of the above autocoids' enzymatic destruction could produce untoward effects on normal fetal development. Actually, this has been proven to be the case for serotonin (10–12), reserpine (13), and iproniazid (14). All have been shown to

produce various adverse effects on embryogenesis including fetal death.

This investigation was therefore undertaken to determine the following: (*a*) to reconfirm in a new strain of mice the lethal and teratogenic effects of serotonin; (*b*) to compare the effects that all three autocoids have on embryogenesis; (*c*) to determine if these agents are directly lethal in half-day-old offspring; and (*d*) to investigate the effects produced on placental transport *via* use of radio-^{24}sodium.

EXPERIMENTAL

CF-1 albino mice[1] were employed in all experimental trials. Males and females, weighing between 25–30 g., were obtained in lots of 100. The females were placed in aggregate cages, 30 females to a cage, while the males were placed in individual cages[2] measuring 12.5 × 15 × 10 cm. All mice were maintained on Purina laboratory chow and allowed access to tap water *ad libitum*.

Serotonin[3] and angiotensin[4] solutions were prepared fresh each week and held under refrigeration when not being used. Synthetic bradykinin,[5] obtained in 1-ml. glass vials, each vial containing 0.1 mg./ml., was also similarly maintained when not in use.

To produce timed pregnancies, two female mice were placed in a male's cage 4:00 p.m. on Monday, Wednesday, Thursday, and Friday. The following mornings, at 7:00 a.m., the females were removed and examined for evidence of a vaginal plug. This plug indicated that the female had been receptive to the male and copulation had occurred. Females in which a plug was observed were regarded as pregnant and this day designated as Day 0 of pregnancy. Females which did not exhibit a plug were returned to the aggregate cage for future breeding.

The pregnant mice were placed in individual cages, similar to the males' cages, and maintained undisturbed, except for drug administration, until the 18th day of gestation.

The experiment was designed so that there were five basic categories: untreated controls, saline controls, serotonin-, angiotensin-, and bradykinin-treated groups. The untreated control mice were utilized to determine the normal spontaneous incidence and type of anomalies for the CF-1 strain of mice. The remaining categories were each subdivided into two smaller groups based on the route of drug administration, *e.g.*, s.c. and i.v. Each of these groups was in turn composed of six smaller groups based on the day of drug or saline administration (0.25 ml.) (Days 7–12). Serotonin (s.c.) was administered at two different dose levels (1 mg./mouse and 10 mg./kg.) while only one dose (10 mg./kg.) was employed in the i.v. groups. Angiotensin II was employed at one dose level in both the s.c. and i.v. groups (10 mg./kg.), while bradykinin was administered, both s.c. and i.v., in a fixed dose of 25 mcg./gravid mouse.

Each mouse, on the 18th day of gestation, was sacrificed by cervical dislocation and the uterine horns surgically exposed. The number of viable fetuses and resorption sites was determined and then the uterine horns were incised exposing the fetuses. The fetuses were removed, blotted dry, and weighed to the nearest tenth of a gram. They were examind for external defects and sexed on the basis of their external genitalia. Every third fetus was prepared for bone examination according to the method of Staples and Schnell (15), while the remaining two-thirds of the litter was placed in Bouin's solution for 2 weeks. After this period, these fetuses were transferred to 70% ethanol for storage, prior to examination of the soft tissues. Before examination for internal soft tissue defects, all specimens were reexamined for external defects under a binocular dissecting scope, (Spencer), after which freehand sections, 1 mm. thick, were made with a thin, double-edged razor blade according to the method of Wilson (16).

In the latter part of this study, each test agent was administered directly to half-day-old offspring, by i.p. injection, using a 0.63-cm. (¼-in.) 26-gauge needle, to determine if these agents were directly toxic. Serotonin, angiotensin, and bradykinin were all administered in a fixed dose of 0.01 mg./fetus, in a volume of 0.1 ml. Saline was administered, 0.1 ml., in a separate test group to serve as a basis of comparison. These groups were composed of five males and five females, randomly chosen from several litters. All offspring were observed for 1 hr. for gross signs of toxicity, and if viable at the end of 1 hr., they were considered to have survived.

In addition, all three test agents were studied for their effect on placental vessels and transport of ^{24}sodium,[6] *in vivo*, employing the procedure described by Robson and Sullivan (17), in 1965. Test groups, each consisting of four gravid mice, 15–17 days pregnant, were designated as A, B, C, and D. Group A was the control which received only the isotope, by i.v. injection, while Groups B, C, and D were, respectively, treated with serotonin (10 mg./kg.), angiotensin II (10 mg./kg.), and bradykinin (25 mcg.), plus 3 μc. of radio-sodium.

In each of the experimental groups, the appropriate drug was administered by intravenous injection, 5 min. prior to the injection of isotope. After the isotope had been administered, the mice were sacrificed, by cervical dislocation, at 5-min. intervals. The thoracic cavity was quickly opened and while the heart was still beating, a blood sample (0.1 ml.) was withdrawn by direct cardiac puncture. This blood was immediately placed in a clean, dry, labeled, and tared glass vial, of a size that would fit the counting well of a gamma-well crystal scintillation counter.[7] Samples of placental and fetal tissue were also removed, aggregated, and placed in separate vials as above.

The activity of each sample was determined by counting each sample twice, for 1 min., and then calculating the average minute count. The sample weights were calculated by reweighing the vials with the samples and then subtracting the tare weight. All weighings were made on a balance (Mettler) to the nearest tenth of a milligram. The results of the counting, plus the weights of the samples, after correction for background and half-life decay, were used in the following formula:

$$\frac{\text{counts/g. tissue/sec.}}{\text{counts/g. maternal blood/sec.}} \times 100 = \%$$

Thus in all experiments, the activity of the samples, and hence their concentration of isotope, were reported as a percentage of the maternal blood levels. The following data were collected for each mouse in the experimental groups: counts/g. tissue/sec., sacrifice time after the injection of isotope, and the activity of the samples expressed as a percentage of the individual maternal blood levels. Then using normal graph paper, the percentage values (*y*-axis) were plotted against the time interval (in minutes) at which the samples were collected. The values obtained for the placentas and fetuses of each litter in an experimental group were plotted on the same graph. Then using these points, the best straight line was fitted and the slope determined. This was done because the slopes represent a convenient way of measuring the entrance rate of the isotope into the placentas and then into the fetuses.

It was anticipated that if the test agents interfered with the transfer of radio-^{24}sodium, the slope of the entrance rate of the isotope into the placenta would be decreased, and if the drug interfered with the transfer of the isotope from fetal placenta to fetus, the second slope would also be decreased, compared to the control values.

Statistical Methods and Analysis—The degree of significance of the observed variations for the experimental groups was determined by the use of standard statistical methods. These

[1] Obtained from Carworth Farms, Inc., New City, N. Y.
[2] Norwich Wire Works.
[3] Marketed by Aldrich Chemical Co., Milwaukee, Wis., as the creatinine sulfate salt.
[4] Supplied through the courtesy of Ciba Pharmaceutical Co., Summit, N. J., as Hypertensin-Ciba (valyl-5 angiotensinamide).
[5] Supplied through the courtesy of Sandoz Pharmaceutical Co., Hanover, N. J.
[6] Obtained from the Iso/serve Division of Cambridge Nuclear Corp., Cambridge, Mass., as isotonic saline, 1 mc./ml.
[7] Baird-Atomic.

Table I—Cumulative $\overline{X}$ Values of Test Groups

Group	N No. Litters	Fetuses R	Fetuses L	Resorptions R	Resorptions L	$\bar{x}$ Fetal Wt.	No. Males	No. Females
Control	26	5.8	4.8	0.07	0.11	1.20	5.24	5.48
S.C.-s.c.[a]	37	4.5	4.3	0.51	0.51	1.18	5.20	4.50
S.C.-i.v.	35	4.7	4.5	0.31	0.37	1.18	5.21	4.63
5-HT-s.c.(1 mg.)	32	0.87[b]	0.53[b]	4.0[b]	3.7[b]	1.03[b]	2.55[b]	2.44[b]
5-HT-s.c.(10 mg./kg.)	35	2.3[b]	2.1[b]	2.7[b]	2.1[b]	1.10	3.60[b]	3.18[b]
5-HT-i.v.(10 mg./kg.)	24				Total Resorption			
A-s.c.[c]	36	5.4	4.3	0.48	0.28	1.12[b]	5.38	4.91
A-i.v.	36	4.8	5.2	0.42	0.28	1.15	5.42	4.94
B-s.c.[d]	36	4.5	4.6	0.6	0.38	1.16	4.73	5.11
B-i.v.	42	4.3	4.2	1.0[b]	0.73	1.05[b]	5.19	4.28

[a] s.c. = subcutaneous. [b] Significantly different from control (S.C.) $p < 0.05$. [c] A = angiotensin II. [d] B = bradykinin.

included the Student t test for continuous variables and the uncorrected (chi square) test for binomial variables. The probability was determined, for the t and chi square values, from standard probability tables.

RESULTS

Gross Maternal Effects of the Drugs—The administration of serotonin s.c., in either of the doses (1 mg./mouse or 10 mg./kg.), and the administration of angiotensin s.c. (10 mg./kg.) did not produce any readily observable effects in gravid mice. Both of these agents, however, when administered i.v., produced several notable effects. Serotonin produced excitation for 1 min. followed by a period of depression lasting approximately 5 min. Angiotensin i.v. administration produced similar effects which were more transient, lasting only 2–3 min. When bradykinin was injected s.c. (25 mcg./mouse), it caused stimulation, increased respiration, and produced apparent pain at the site of injection for several seconds. Bradykinin, when injected i.v., caused more pronounced stimulation, and even outright convulsions in a few cases, and pain which caused the mice to squeal.

In no case did the s.c. or i.v. administration of the test agents cause death in the teratogenic studies; however, in the radio-sodium experiment, one of the gravid mice died immediately after the i.v. injection of angiotensin.

Mean Number of Viable Fetuses—From Table I, it can be seen that the control group, comprised of 26 litters, had a mean of 5.8 fetuses in the right horn and 4.8 in the left. The saline s.c. and i.v. test groups, which served as controls for the different routes of administration of the test drugs, had means of 4.5 and 4.7 fetuses, respectively, in the right horn while the left contained the respective means 4.3 and 4.5. Furthermore, it can be seen from Table I that only serotonin when administered subcutaneously, in either dose, significantly affected the mean number of viable fetuses in the right and left uterine horns, causing a reduction in both. In the i.v. trials, serotonin caused complete resorption of all litters while angiotensin i.v. and bradykinin i.v. had no adverse effect on the number of viable fetuses.

Intrauterine Deaths, Resorptions and Mean Fetal Weight—As would be expected, all agents which reduced the mean number of viable fetuses present in the uterine horns concomitantly increased the number of fetal resorptions present.

Table II—Summary of Anomalies

Group	Anomaly	Incidence (No. Fetuses)	Incidence Anomaly, %	Total Anomaly, %	Abnormalities (χ^2)[a]
Control	Exencephalus	2	0.75	1.5	—
	Gastroschisis	1	0.37	—	—
	Cryptorchidism	1	0.37	—	—
Saline control s.c.	Hydronephrous	1	0.26	0.26	—
Saline control i.v.	Hydronephrous	1	0.26	0.26	—
5-HT-s.c.(1 mg.)	Gastroschisis	2	4.4	—	—
	Hydrocephalus	3	6.6	—	—
	Hydronephrous	2	4.4	24.4	83.78
	Open ear	1	2.2	—	—
	Anophthalmia	1	2.2	—	—
	Enlarged liver	1	2.2	—	—
	Exencephalus	1	2.2	—	—
5-HT-s.c.(10 mg./kg.)	Gastroschisis	7	4.4	—	—
	Hydrocephalus	10	6.2	—	—
	Hydronephrous unilateral	2	1.2	27.0	91.95
	Hydronephrous bilateral	22	13.7	—	—
	Renal agenesis	2	1.2	—	—
5-HT-i.v.(10 mg./kg.)	Total resorption	—	x	x	x
A-s.c.(10 mg./kg.)	Microphthalmia	1	0.28	—	—
	Nasal septum defect	1	0.28	1.2	0.771
	Testicular agenesis	1	0.28	—	—
	Anophthalmia, rt.	1	0.28	—	—
A-i.v.(10 mg./kg.)	Fusion of sternebrae	1	0.27	0.27	0.006
B-s.c.(25 mcg.)	Hydronephrous	2	0.58	—	—
	1-Miss. sternebra	1	0.29	0.87	0.100
B-i.v.(25 mcg.)	Hydronephrous, rt.	4	1.13	—	—
	Hydronephrous, lt.	6	1.69	—	—
	Hydronephrous B.	15	4.24	—	—
	Delayed ossification[b]	18	5.09	12.7	34.16
	Inc. cer. vertebrae	1	0.283	—	—
	Supraoccipital plate defect	2	0.56	—	—
	Extra sternebrae	1	0.28	—	—
	Rib fusions	2	0.56	—	—

[a] Significant (χ^2) > 3.84 = significantly different from control. [b] Cranial plates.

From Table I, it can be seen that serotonin, when administered in either dose subcutaneously, increased the number of fetal resorptions ($p < 0.001$), while the i.v. administration of this agent caused total resportion of all fetuses. Subcutaneous angiotensin and bradykinin and i.v. angiotensin did not significantly ($p > 0.05$) alter the resorption rate; however, the i.v. administration of bradykinin increased the resorption rate only in the right uterine horn.

Moreover, from this table, it can be observed that the s.c. administration of 5-HT 1 mg./mouse reduced the mean weight of the fetuses as did the s.c. administration of angiotensin. When administered i.v., serotonin caused complete resorption and bradykinin reduced the mean fetal weight, but the mean fetal weight remained unaltered in the other test groups.

Production of Anomalies—Types and Incidence—The types of defects obtained and their incidences are presented in Table II.

The greatest incidence of anomalies occurred on Day 8 of gestation with serotonin (1 mg./kg.) and Day 11 with the 10 mg./kg. group (Fig. 1). Angiotensin s.c. and i.v. produced the highest incidence of anomalies on Days 8 and 11, respectively, while bradykinin s.c. and i.v. produced the highest incidence on Days 10 and 8, respectively (Fig. 2).

Results of Radio-Sodium Investigation—The four mice which comprised the control group were sacrificed at 5, 10, 15, and 20 min., respectively, after the i.v. administration of the radio-sodium. Three tissue samples were collected from each mouse (blood, placental, and fetal). Their counting rates were determined and corrected for background, half-life decay, and tissue weight to give counts/g. tissue/sec. The percent activity of the placental and fetal samples were calculated using their respective blood sample level as 100%.

The placental values for the 5-, 10-, 15-, and 20-min. animals were respectively determined to be 21.9, 24.6, 43.2, and 15.3%, while the fetal values were found to be 7.2, 5.9, 15.1, and 10.1%, respectively. The percent activity values for placental and fetal samples were than plotted against elapsed time, and the best straight line fitted to the first three points. The entrance rate of the isotope into the respective tissues was then determined by calculation of the slopes of these two lines. These values were found to be 3.0 and 0.93, respectively, for the placental and fetal samples. This indicated that the isotope entered the placental tissue approximately three times faster than that of the fetus.

Figure 1—*Typical gross defects, upper left to right: first two fetuses, untreated controls demonstrating exencephalus; third, serotonin-treated exencephalus; fourth, serotonin-treated gastroschisis; lower, normal fetus for comparison.*

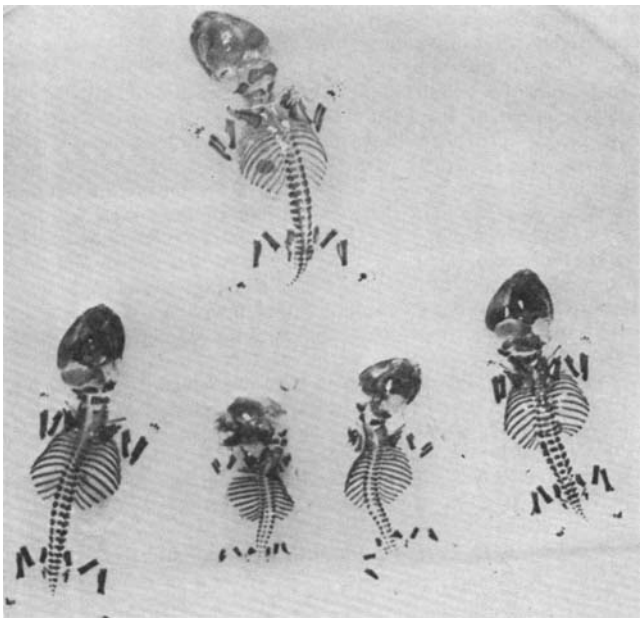

Figure 2—*Typical skeletal defects occurring in the bradykinin i.v. test group: upper, normal fetus; lower left to right: first fetus, normal size but retarded fore and hindpaw ossification; second and third fetuses, retarded growth and marked retardation of fore and hindpaw ossification; fourth fetus, normal size and paw ossification but demonstrating fusion of the last three ribs.*

Serotonin Treated—The same procedure was followed in this group as in the control, except that the mice were all injected i.v. (*via* tail vein) with serotonin (10 mg./kg.) 5 min. prior to the administration of the isotope.

The placental values (percent activity of blood level) were determined to be 8.2, 8.9, 30.4, and 19.3% for the respective time intervals. The fetal values were found to be 1.8, 2.3, 3.7, and 18.0%, respectively. These values were then plotted, as described in the control, and the placental and fetal entrance rate of isotope determined to be 1.49 and 0.25, respectively.

Angiotensin-Treated—Like the serotonin group, these animals were pretreated with the test drug, in this case, angiotensin II (10 mg./kg.) i.v. before the injection of isotope.

The placental and fetal samples were determined to have the respective percent activity values of 18.2%, 28.2%, died, and 38.2% for the placentas and 3.9%, 5.4%, died, and 16.3% for the fetal samples. These values were plotted and the entrance rates determined to be 2.5 and 0.53, respectively, for placental and fetal samples.

Bradykinin-Treated—The mice were pretreated, 5 min. prior to the isotope administration, with 25 mcg. of bradykinin i.v. Tissue samples were collected at the appropriate times and the percent activity of the placentas and fetuses was determined to be 35.2, 48.1, and 43.6% for the placentas, and 3.7, 9.3, and 11.6% for the fetal samples. These values were plotted and the placental and fetal entrance rates found to be 4.24 and 0.80, respectively.

DISCUSSION

Serotonin, administered s.c., in either dose, as well as s.c. angiotensin failed to produce observable effects in gravid mice. However, whenever these agents were administered intravenously, they produced a short period of excitation, which was then followed by a period of depression lasting approximately 5 min. for serotonin and only 2–3 min. for angiotensin. The stimulation produced by these agents was similar but longer in duration to that produced by the saline i.v. injection. In the case of i.v. serotonin, the stimulation observed was similar to that reported (18) for other routes of administration with large doses. Stimulation by angiotensin was probably due to injection trauma and blood pressure elevation. Any depression

observed after stimulation by serotonin and angiotensin was considered after-depression which ordinarily follows stimulation. The stimulation observed with the administration of bradykinin, *via* either route of administration, can probably be attributed to its ability to produce pain sensations; the convulsions produced may have been due to a sharp drop in blood pressure (19) before compensation occurred. A more profound after-depression occurred following the i.v. administration of bradykinin than with either of the other agents.

The experimental administration of drugs can affect the number of viable fetuses in many ways. They may interfere with ovulation, fertilization, implantation, hormonal levels responsible for maintenance of pregnancy, the proper nutrient supply to the fetus, or cross the placental barrier and produce direct toxic effects.

From Table I, it can be seen that the control group as well as all of the experimental groups, except for A-i.v., had a higher number of fetuses in the right horn than in the left which supports the work of McLaren (20), who demonstrated that, in mice, the right uterine horn tends to be superior to the left in respect to ovulation and survival of embryos. It is also apparent from Table I that all of the experimental groups had fewer fetuses present than the untreated controls. This is probably due to the trauma of handling and injection which has been shown by Runner and Jackson (21) to produce such an effect. In the treated groups, of course, the reduction in mean viable fetuses can be attributed to both handling and drug effects. Furthermore from Table I, it can be seen that only serotonin s.c. and i.v., in either dose, significantly lowered the mean number of viable fetuses compared to the saline control values, thus indicating a definite toxic effect. The fact that serotonin is lethal to embryos has been shown in several investigations (22–24). These investigations have shown that serotonin can terminate gestation by affecting hormonal levels and by a direct effect on uterine contents. It is presently believed that it interferes with the proper nutrition or oxygen supply of the developing embryos, thereby causing intrauterine deaths.

From Table II, it can be seen that the untreated control had an anomaly incidence of 1.5%, which included exencephalus, gastroschisis, and cryptorchidism, while both saline groups (s.c. and i.v.) had only one type of anomaly occur, hydronephrous; 0.26% incidence for both. The reason for a lower incidence in these control groups as well as the occurrence of a different anomaly compared to the untreated group cannot be explained at the present time. The serotonin-treated group (s.c. 1 mg./mouse) produced a 24.4% incidence of defects. Some of these defects were similar to the untreated and saline control groups, for all demonstrated an incidence of exencephalus, gastroschisis, and hydronephrous. In addition, treatment with serotonin 1 mg./mouse produced several different anomalies, which had not occurred in the controls, namely: hydrocephalus, open ear, anophthalmia, and liver enlargement. The incidence of all anomalies was much higher than the controls. When serotonin was administered at the lower dose (10 mg./kg.), subcutaneously, it produced a higher incidence of defects than the 1-mg. dose, which can be attributed to its lesser toxicity and greater fetal survival. The defects that occurred were similar to the 5-HT-1-mg. group, gastroschisis, hydronephrous, hydrocephalus, and one additional type, renal agenesis. Defects that the authors observed in the serotonin-treated groups were in good agreement with those reported by Reddy *et al.* (10), who produced anophthalmia, hydrocephalus, exencephalus, and exomphalos, a defect related to gastroschisis. Hence this experiment has confirmed the teratogenic effects of serotonin in a new strain of mice.

Defects that were observed in the angiotensin groups, even though of such a low incidence as to be not significantly different from the control group were of a different type than that observed in the saline control and the untreated control, namely: microphthalmia, a nasal septal defect, testicular agenesis, and right anophthalmia. This indirectly may indicate that this agent is a weak teratogen.

Bradykinin, when administered s.c., as seen in Table II, resulted in the production of 0.87% incidence of anomalies, which did not represent a significant difference from the s.c. saline control group. Two types of defects occurred, namely: hydronephrous and one fetus with a missing sternal ossification site. However, when bradykinin was administered by i.v. injection, it did produce a significant increase in anomalies and also some new types. It produced hydronephrous similar to the controls but to a much greater degree, defects of the supraoccipital plate, generalized retardation of ossification, incomplete cervical vertebrae, rib fusions, and (not listed in the tables) a pronounced reduction of the number of ossification sites in the fore- and hind-paws. This number was reduced approximately 50% compared to the saline i.v. control (means of 13.1 for the control and 7.1 bradykinin-treated).

The tracer experiment was performed to determine whether the test agents, serotonin, angiotensin, and bradykinin could affect the transport of radio-^{24}sodium (^{24}Na) from the maternal circulation into the placenta and then into the fetuses. This particular isotope was chosen because it has been purported to be a good indicator for determining placental function especially with regard to transfer (17). It was imperative to determine if these agents affected placental transfer, for if they did, it could account for the teratogenic effects observed.

Factors which affect the rate, degree, and selectivity of materials transported or transferred across the placental membrane and then into the fetus can be summarized as follows:

maternal blood ..... placenta ..... fetus
A B

The factors involved in transport at Point A (from maternal blood to fetal side of the placenta) are: maternal blood concentration and availability of isotope; maternal blood flow; passive diffusion; and active transport.

The factors involved in transport at Point B (fetal side of placenta to fetus) are: placental isotope concentration; placental blood flow; umbilical vessel tonus; and fetal viability.

The first four factors, affecting transport at A (from the maternal blood stream across the placental barrier into the fetal side of the placenta), are extremely complex and highly interdependent on one another. For example, a drug which causes vasoconstriction or dilation affects transfer of the isotope by changing maternal uterine blood flow which, of course, would change the local isotope concentration and availability which would then affect passive diffusion. If the test drug was able to produce intense constriction of the uterine vessels, it may cause local tissue hypoxia or anoxia which could affect active transport mechanisms. A decrease in maternal blood flow could cause a local increase in placental p CO_2 that may affect the permeability of the fetal membranes, thereby affecting the transfer of isotope. This local increase in p CO_2, and possibly the accumulation of other waste products, due to the decreased maternal uterine blood flow, could conceivably alter, stimulate, or depress fetal circulation by affecting umbilical vessel tonus, or cardiac rate and output. This may then affect the transfer of isotope from placenta to fetus. All changes that occur at Point A will probably affect the transfer at Point B (from fetal placenta to fetus) either directly or indirectly. The administered drug, if it crosses the placental barrier, may directly affect the fetal circulatory system, *e.g.*, cardiac rate and output, which as previously mentioned could alter the transfer of isotope.

The control values indicate that the transfer of isotope from the maternal blood into the fetal side of the placenta proceeds at a rate approximately three times that of the transfer from placenta to fetus, the respective values being 3.0 and 0.93. It was determined that the prior administration of serotonin (10 mg./kg.) i.v. to gravid mice greatly decreased the transfer of isotope from the maternal blood to the fetal side of the placenta and that the transfer from this point to the fetus *per se* was drastically reduced. This was determined on the basis that the placental entrance rate was reduced 50% compared to the control value (1.49 as compared to 3.0); while the fetal transfer rate was reduced approximately 75% compared to the control value (0.25 compared to 0.93). This reduction in transfer of isotope could be due to interference with any or all of the previously mentioned factors. However,

in view of the fact that serotonin has been shown to be a potent constrictor of fetal placental vessels (1), and also the umbilical vessels (25), it would seem likely that these are two major factors contributing to the reduced transfer. The constriction may cause sufficient reduction in fetal circulation through the placenta to allow the accumulation of CO_2 and other waste products, which may account for teratogenesis since Haring (26), in 1960, proved that exposure of gravid rats to high concentrations of carbon dioxide produced cardiac anomalies in the offspring. Serotonin has been shown to reduce the uterine blood flow to the placenta (17), which consequently will decrease the availability of the isotope for transfer and may cause a local deficiency of oxygen. This is pertinent, for the placenta has been shown to have a high oxygen consumption rate associated with its active transport of materials (27). It appears likely that if the oxygen supply to the placentas is lowered, it could interfere with proper functioning of the active transport mechanisms, and hence transfer of isotope. This reduction in uterine flow may account for the observed defects, for Brent and Franklin (28) have shown that an impairment of uterine vascularization in rats resulted in retarded growth, malformations, and even death of the offspring. Ingalls *et al.* (29) have also demonstrated that a reduction in available oxygen can produce various heart defects in the offspring. The interrelationship between hypoxia and increased CO_2 in the production of heart defects is particularly interesting in view of the fact that Robson and Sullivan (17) have demonstrated that a single injection of 2 mg. of serotonin in mice caused death of the fetuses within 0.5 hr., which was due to fetal cardiac arrest. It was noted in the present investigation that when the placental samples were collected for radio assay, they were very dark, almost black in color, probably due to poor circulation with concomitant decreased oxygenation.

Another factor which may contribute to the decreased transfer of isotope from the maternal blood to the fetal side of the placenta is a direct effect of serotonin on the permeability of the placental barrier, since Pickles (30), in 1956, has demonstrated that serotonin alters membrane permeability. This, of course, could affect the transfer of nutrients or oxygen which may be responsible for the observed teratogenic effects.

The results obtained for the angiotensin-pretreated mice indicate that this agent is also capable of reducing the transport of radio-sodium. This was shown by an approximate 17% reduction in maternal blood to fetal placental transfer (2.5 compared to 3.0) and a 43% reduction in placental to fetus transfer (0.53 compared to 0.93). This appears to be due mainly, in view of the larger reduction of placenta to fetus transfer where passage through the placental barrier is not involved, to vasoconstriction of the vessels on the fetal side of the placenta and possibly the umbilical vessels. This is further supported by the fact that angiotensin has been shown *in vitro*, to cause constriction of these vessels (31). The slight decrease on the maternal side may be due to constriction of the maternal vessels supplying the placentas or the uterine vessels. The placentas, when removed for radioassay, were noted to be normal in color and gross appearance, unlike the serotonin-treated ones.

The bradykinin group demonstrated a 41% increase in isotope transfer from maternal blood to fetal placenta. This could be the result of increased maternal blood flow, due to vasodilation of the maternal vessels supplying the uterus and the placentas, or it may represent an increase in the total volume of the maternal placenta or a direct effect of bradykinin on the permeability of the placental membranes. Bradykinin has been shown to be a potent vasodilator thereby supporting the concept that this increase may have been due to increased maternal blood flow. However, alteration of membrane permeability is probably just as, if not more, important in accounting for the increased transfer of sodium, because bradykinin has been shown to be 10 to 15 times more potent than histamine in regard to increasing capillary permeability (19). This may therefore, from the teratogenic aspect, be the most important factor, for the increased permeability could allow materials to cross the placental barrier which normally would be excluded. The slight decrease in the transfer of sodium from fetal placentas to fetuses could be due to bradykinin's ability to constrict the fetal placental vessels (1).

There is one other factor that could account for a decrease in entrance rates, and this is fetal death. However, this was not a contributing factor in any of the drug-treated groups because all of the fetuses were observed to be viable when tissue samples were collected.

The agents which produced significant teratogenic effects, serotonin and bradykinin (i.v.), drastically altered the entrance rate into the fetal side of the placenta, while the third agent, angiotensin, which did not produce significant defects, only slightly reduced this rate. Furthermore, serotonin and angiotensin definitely reduced the transfer from placenta to fetus, while bradykinin did not cause much of a reduction. Thus, although they all appear to cause placental vasoconstriction, it becomes apparent that the common denominator for the two agents which produced birth defects was alteration of transfer across the placental membranes. The fact that angiotensin reduced transfer into the fetus, like serotonin, yet did not produce defects, and the fact that bradykinin did not affect this to any great extent indicates that alteration of this second transfer was not the one responsible for the observed teratogenic effects.

In conclusion, it appears that any agent which can alter, directly or indirectly, the permeability or transport mechanisms of the placental barrier, thereby interfering with the transfer of oxygen or nutrients or allowing substances to cross the barrier which normally do not, may be responsible for the production of adverse effects during embryogenesis.

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Viscoelastic Properties of Pharmaceutical Semisolids I: Ointment Bases

S. S. DAVIS

Abstract □ The rheological evaluation of semisolids by continuous shear methods is limited in its application. However if materials are viscoelastic, their properties can be assessed in creep testing where fundamental parameters are provided and rheological behavior can be represented by mechanical models. Measurements have been made on a number of ointment bases over a range of temperature. The addition of small quantities of complex materials to paraffin ointment bases changes their behavior from elastic to viscoelastic. As many of the materials show a characteristic change in viscoelastic spectrum with temperature it is hoped that creep testing will provide a suitable method for the correct rheological evaluation of many important materials.

Keyphrases □ Viscoelastic properties—ointment bases □ Ointment bases—creep testing □ Creep compliance—ointment □ Temperature effect—viscoelastic properties □ Shear, continuous, creep—ointment viscoelasticity

The rheological evaluation of pharmaceutical semisolids is useful in that it provides both a method of quality control during and after manufacturing processes (1–3) as well as information as to the structures of the phases present in a material and the influence of various agents used in its formulation. Continuous shear-rheology has been a popular approach (4, 5) and in particular the Ferranti-Shirley viscometer, with automatic flow-curve recording unit, has been much utilized (6–10). However many pharmaceutical semisolids demonstrate complex rheological behavior that is difficult to characterize in this way (11). If the semisolids are viscoelastic in nature it is more valuable, both theoretically and practically, to examine them in their rheological ground state where the method of testing does not significantly alter the structure.

The importance of viscoelasticity in pharmaceutical materials has been discussed briefly by McVean and Mattocks (12) and Berneis and Munzel (13). A detailed study of the viscoelastic system, sodium lauryl sulfate, cetyl alcohol, water (with and without the addition of oil) has been made by Barry (6, 14). The theory of linear viscoelasticity (15) can be applied to pharmaceutical materials (2, 16) and recently a number have been examined by oscillatory methods.

One of the simplest methods of examining viscoelasticity in a semisolid is the creep test where a stress is suddenly applied and then maintained constant, sometimes for a considerable period of time. The time-dependent strain response is known as the creep curve. If the strain is in the linear region, where the ratio of stress to strain is a function of time alone and not strain magnitude (17), the creep curve can be analyzed using the theory of viscoelasticity. The strain response on removal of the stress is know as the recovery curve. A typical creep curve is shown in Fig. 1. The creep curve can be split up into three separate regions each of which can be represented by a mechanical model (18). The region A-B represents an instantaneous elastic component which can be associated with an uncoupled Hookean spring. Region B-C is where the flow is viscoelastic and can be represented by a Voigt unit(s). This is a spring in parallel with a dashpot. Region C-D is that of viscous flow and can be associated with an uncoupled residual Newtonian dashpot. In recovery, the regions A-B, B-C, are recov-

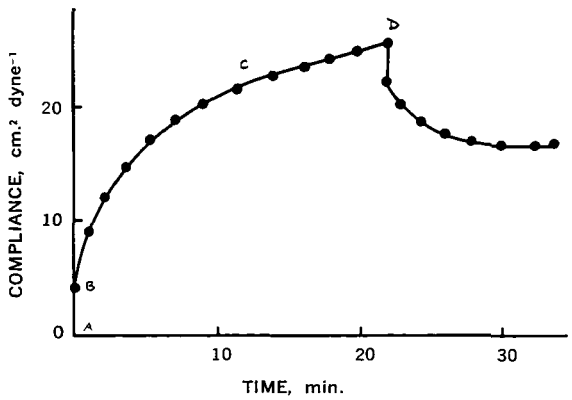

Figure 1—*Typical creep curve for a viscoelastic material. A–B, instantaneous elastic region; B–C, Voigt region; C–D, region of viscous flow.*