OKLAHOMA JUNIOR ACADEMY

SELECTED PAPERS

A Study of the Effects of Six Drugs on Chick Embryo Development

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INTRODUCTION

In recent years many drugs have been removed from the market because of adverse side-effects. Sandostene, an antihistamine, destroyed blood cells; and Flexin, a muscle relaxant, caused extensive liver damage. It is possible that Thalidomide will be remembered as the most tragic drug in medical history. The disclosure in 1962 that this drug, which had been called the "perfect hypnotic," could maim and destroy the unborn emphasized the lethal possibilities of synthetic drugs. Thalidomide victims suffered a variety of abnormalities. The most common deformity was short, deformed, useless arms (phocomelia). Others were deformities of the legs, feet, ears, digestive tract, heart, and blood vessels.

These findings prompted a study of the effects of some drugs on chick embryo development. The original intent was to test drugs that had not yet been released for public use, but working with such drugs by a minor is prohibited by law. Consultation with a pharmacist resulted in the use of more assessible materials.

DRUGS USED

The drugs used were common drugs that might be found in any household. All but Drug D and Drug F are available without prescription. All drugs used were related through the analgesic properties. A description of each drug and its effects on humans follows.

Drug A (Aspirin)

Description. Each white tablet contains:

Acetylsalicylic acid ..... 5.0 gr

Pharmacology and Chemistry. Acetylsalicylic acid is the most commonly used drug in the world. It relieves headache, neuralgia, muscle and joint pain and is used in the treatment of rheumatic fever. Drug A reduces fever.

Side-Effects. The most common side-effect is gastric upset. Excessive use of acetylsalicylic acid can cause salicylism, a syndrome characterized by tinnitus, dizziness, blurred vision, sweating, skin eruptions, nausea, and vomiting. Intensive use of this drug can reduce the prothrombin level in the blood to the point where severe bleeding can occur.

Drug B (Excedrin)

Description. Each white tablet contains:

N-acetyl-p-aminophenol
Salicylamide
Acetylsalicylic acid
Caffeine

Pharmacology and Chemistry. Drug B is merely a strengthened form of Drug A. It relieves headache, arthritis, sinusitis, colds, and toothache.

Acetylsalicylic acid and salicylamide have similar structures the molecules in each being modified benzene rings.

Side-Effects. The most common side-effect is gastric upset.

(Information is lacking about Drug B because the manufacturer did not respond to requests for information.)

Drug C (Triaminicin)

Description. Each capsule-shaped, slender, yellow tablet contains:

Phenylpropanolamine hydrochloride	25.0 mg
Pheniramine maleate	12.5 mg
Pyrilamine maleate	12.5 mg
Acetylsalicylic acid	225.0 mg
Acetominophen	150.0 mg
Caffeine	30.0 mg
Ascorbic acid	50.0 mg

Pharmacology and Chemistry. In colds, nasal allergies, sinusitis, rhinitis, and post-nasal drip, Drug C decongests and normalizes respiratory passages, eliminates irritating secretions, combats allergic symptoms, and improves sinus drainage. The analgesic combination in Drug C resembles the *APC* formula which has been used for the relief of pain from headache, neuralgia, the common cold, rheumatic conditions, and tooth extraction.

Side-Effects. Drowsiness, blurred vision, cardiac palpitations, flushing, dizziness, nervousness, and gastrointestinal upsets may occur.

(Information courtesy of Dorsey Laboratories.)

Drug D (Darvo-Tran)

Description. The pink and red capsules contain:

Propoxyphene hydrochloride	32.0 mg
Acetylsalicylic acid	325.0 mg
Phenogylcodol	150.0 mg

Pharmacology and Chemistry. Propoxyphene hydrochloride (a-d-dimethylamino-1, 2-diphenyl-3-methyl-2-propionoxybutane hydrochloride) is the only one of a group of four stereoisomers which possesses analgesic activity. It is a white crystalline solid which is soluble in water.

Phenoglycodol is 2-p-chlorophenyl-2-methyl-2, 3-butanediol. Phenoglycodol has a completely different structure than most tranquilizers. (Most tranquilizers are modeled after codine and morphine which have three benzene rings.) It has low solubility in water.

Drug D effectively relieves arthritic conditions, tension, headache, low-backache, whiplash injuries, oral surgery procedures, and post-operative pain. It is used also when emotional factors intensify pain or if immediate relief is not available.

Side-Effects. Therapeutic doses have produced no alarming side-effects. The most frequent adverse effects have been referable to the gastrointestinal tract. Nausea, gastric irritation, and emesis have been noted. These particular effects occur commonly with the use of acetylsalicylic acid. For this reason it is difficult to pinpoint the offending drug. Other side-effects reported are vertigo, drowsiness, and/or excitation.

(Information courtesy of the Eli Lilly Company.)

Drug P (Painquilizer Tablets)

Description. Each white and pink, two-layered tablet contains:

Dihydroxy Aluminum Sodium Carbonate
Magnesium Carbonate
Acetophenetidin
Caffeine

Pharmacology and Chemistry. Drug P brings symptomatic relief from pain of headache, neuritis, neuralgia, and colds. It relieves minor pain of arthritis and rheumatism and corrects over-acid stomach. Acetophenetidin is relatively toxic.

Side-Effects. Overdose will cause nausea.

(Data is lacking about Drug "P" because the manufacturer did not respond to requests for information.)

Drug F (Phenophen)

Description. Each brown and white capsule contains:

Phenacetin	194.0	mg
Acetylsalicylic acid	162.0	mg
Phenobarbital	16.2	mg
Hyoscyamine sulfate	.031	mg

Pharmacology and Chemistry. Drug F relieves intensive pain and serves as a mild sedative. Its outstanding component is phenobarbital. Phenobarbital (as are all barbiturates) is derived from barbituric acid. The fundamental structure of all barbiturates is R=R'—H. The potency and toxicity of the barbiturates are proportional to the size of the R and R' groups. Optimal properties are achieved when the sum (R+R') is comprised of from six to ten carbon atoms (Sicé, 1962).

Side-Effects. Phenobarbital can be turned into a toxic or lethal drug by a diseased liver or kidney. Barbiturates are addicting. Withdrawal symptoms include an inordinate craving for the drug, tremors, excitement, and sometimes convulsions. Sudden withdrawal may cause death. Hyoscyamine depresses the central nervous system. It is often used in combination with barbiturates and narcotics to produce obstetric amnesia (light sleep).

THE CHICK EMBRYO

The chick embryo is one of the most satisfactory animals on which student laboratory work may be based. Since the observations of the chick embryo by Malpighi (1672), Wolff (1759), and von Baer (1828), it has become the most thoroughly understood and described of all embryos. It is available all over the world at all seasons of the year and can be studied with even the most modest equipment. The following definitive descriptions (Lillie, 1908) are helpful in relating subsequent observations in terms of standard stages:

"Stage 22 (3½-4 days)

1. Limbs: Elongated buds, pointing caudal.
2. Somites: Extend to tip of tail.
3. Flexures: The dorsal contour of the trunk is a straight line or curved.
4. Visceral arches: Maxilary process enlarged; 4th cleft distinct as a slit.
5. Allantois: Variable in size; extends to head and may overlap the forebrain.
6. Eye Pigmentation: Distinct.

Stage 36 (10 days)

1. Limbs: Distal segments of both wing and leg are proportionately much longer.
2. Visceral arches: Primordium of the comb appears as a promi-

nent ridge with slightly serrated edge. The labial groove is clearly visible at the tip of the upper jaw but is barely indicated on the mandible. Nostril has narrowed to a slit.

3. Feather germs: All are conspicuous.

4. Eyelids: Lower lid had grown upward to the level of the cornea. The circumference of lids is a narrowing ellipse with its ventral edge flattened."

PROCEDURE

The reading of literature began in June, 1964; the laboratory work began in late summer. There was some difficulty in locating a supply of fertile eggs at that season of the year, because most flocks were moulting. After building the incubator, a plywood box 24" x 15" x 9" equipped with a thermostatically controlled heat element, the classic exercise of setting 21 eggs and opening one egg each day to observe development was performed. This exercise also gave experience in handling the embryos.

The original experimental approach (unsatisfactory) had been to explant the embryo into a moisture chamber consisting of a watch glass placed in a ring of moist cotton in a petri dish. The medium in the watch glass would have contained the drug. The high mortality rate of the embryos made another method advisable.

The final method was quite simple. An egg of 48 hours incubation was placed in a bed of cotton in a fingerbowl. A piece of shell about one-half inch square was sawed through with the point of a scalpel and removed. Every effort was made to avoid damaging the shell membranes during the process. All small particles of shell were brushed away, and the shell around the opening was wiped with a cotton swab soaked in 1% solution of iodine in 95% alcohol. The membrane was punctured with a sharp needle and cut away.

Experimental Groups. The injection was made into the yolk with a hypodermic needle. Care was taken not to injure the embryo or the vitelline (blood) vessels which cover the vitelline membrane. Each egg received 20mg of a drug dissolved in 0.1cc of distilled water. The fluid was heated to 40°C before injection to avoid shocking the embryo.

A clean circular coverslip was placed over the opening in the shell. A ring of paraffin was painted on the outer edge of the coverslip to keep air from entering the egg abnormally. The egg was returned to the incubator. At 12-hour intervals the egg was rotated about 90 degrees. Care was taken that neither the embryo nor any of the extraembryonic tissues came into contact with the observation window. The chick could be easily observed through the window for several days after the injection date.

The eggs were usually opened at 10 days unless the embryo hemorrhaged or died, in which case the egg was opened at the time the condition was observed. The egg was cracked and the contents were dumped into a fingerbowl containing Locke's solution (Rugh, 1948). After the chick embryo was observed in this solution, it was lifted into a watch glass for further study and finally was either discarded or preserved in 10% formaldehyde.

Control Groups. Three control groups were used. One group was injected using the regular method with plain distilled water. This served as a control for the carrier. The second group received no injection, but the observation window was made in the egg. The third group was allowed to develop normally without a window or an injection. When an abnormal chick is compared to a control chick, the control is of this third group.

TABLES OF RESULTS

Drug Used	Number Injected	Number Abnormal	Number Normal	Number Infertile	Number Dead
Drug A	40	1 (small)	20	7	12
Drug B	20	—	12	6	2
Drug C	40	—	—	8	32
Drug D	40	20 (hemorrhaged)*	4	6	10
Drug P	20	4 (head position)**	12	2	2
Drug F	40	32 (small, underdeveloped)***	—	2	10
H_2O (Control 1)	20	—	12	4	4
None (Control 2 & 3)	20	—	16	3	1

*Embryo hemorrhaged in the vitelline vessels.

**Head grew over shoulder instead of tucked to breast.

***Embryos were in Stage 22 which should have been in Stage 36.

Percentage of Analysis Based on Above Table

Drug A — 90% normal; 10% smaller than control
Drug B — 100% normal
Drug C — 100% fatal
Drug D — 83% hemorrhaged; 17% normal
Drug P — 75% normal; 25% abnormal head position
Drug F — 74% abnormal; 26% fatal

Figures for Drugs A, B, D, and P do not include chicks killed by injection. All cases are included in figures for Drugs C and F.

DISCUSSION

Drugs A and B did not affect the development of the embryos. The slight irregularity in those chicks injected with Drug A is accredited to an error in technique. Even though the syringe and needle were carefully washed and sterilized between injections, it is assumed that some foreign particles were injected into the eggs. There is a very small chance that the embryos were deformed because of some other factor, such as hypersensitivity or an individual idiosyncrasy.

The heartbeat of embryos injected with Drug C stopped within 48 hours of the injection. The first assumption was that the drug was given in too large amounts, but the mortality rate continued when the amount was reduced to 10mg and finally to 5mg. (Twelve eggs were injected with each of the reduced amounts, and 100% of the embryos died in less than 48 hr.)

Drug D caused the embryos to hemorrhage in the vitelline vessels. Though it is a tranquilizer, one of the side-effects of the drug is excitation causing an increase in blood pressure. Perhaps the blood pressure in the embryonic circulatory system is increased and the vitelline vessels, which are still in the formative stages, cannot stand the pressure and break.

The abnormality (head over shoulder) which occurred in 25% of the embryos injected with P cannot be explained. The embryos developed normally in all ways except that the head, rather than being tucked to the breast, was twisted over the shoulder. Careful dissection did not reveal any abnormal internal development.

Drug F caused a constant and decided retardation in growth and development. Chicks of 10 days incubation which should have been in Stage 36 were still in Stage 22. This deformity was probably caused by phenobarbital or hyoscyamine sulfate or a combination of the two. This is assumed because the other two components, phenacetin and acetylsalicylic acid, were contained in other non-deforming drugs.

CURRENT RESEARCH

At the present time, the effects of the individual components of Drugs C, D, and F are being assayed on chick embryos.

All of the component drugs have been acquired. Currently, caffeine, phenogylcodol, and phenobarbital are being used. Only a few drugs can be tested at a time because of limited incubator space.

Caffeine administered in doses of 5mg in 0.1cc of distilled water causes death. The rate of heartbeat increases rapidly for about 4 min, after which it begins slowing irregularly until it stops within 15 min. This effect has been observed on 12 out of 12 embryos. Plans are to use even smaller doses to see how much it takes to effect the embryo and what will happen if the embryo lives. This effect of caffeine is a possible explanation for the high mortality rate of chicks injected with Drug C.

Embryos injected with phenoglycodol have developed, in four out of twelve cases, a condition similar to encephalocoele or herniation of the brain through a defective cranial roof. It has not been possible to determine as yet whether the protruding brain is merely part of the brain that was not properly enclosed in the brain case or whether it was a twinning effect and part of the brain had been developed twice—once in the brain case and once on the outside—although the phenomenon is under study.

Phenobarbital retards growth by about two stages when administered in doses of 15mg in 0.1cc of water. When given in smaller doses (5mg in 0.1cc), the embryos tend to develop in abnormal positions; but they follow no pattern. A leg is often wrapped around the head, but the twisted neck occurs frequently also.

CONCLUSIONS

Obviously, drugs did affect the development of the chick embryos; but limited data prevents significant specific inferences concerning the individual drugs. One conclusion is that all people should be aware that all drugs, even common ones, affect cell behavior. Because of this, no drug should be taken aimlessly.

Much more research is needed in this field. While Thalidomide caused phocomelia, another common drug may be causing some very common afflictions—like myopia. Of five leading drug companies contacted for information about the tests run on drugs before they are put on the market, *none* test drugs for their effects on the development of embryos. This could be a serious deficiency, for, as has been shown, even common drugs have serious effects on embryonic development.

Relationship to Humans. This poses one last question: How do the results of this experiment on chicks relate to mammalian embryological development? In the human being, or any mammal, the developing egg attaches itself to the wall of the birth canal, or womb, designed to hold the developing embryo. The placenta is a mass of tissue that covers the inner surface of the womb. This placenta is infiltrated with blood vessels developed by the embryo, while the womb is equally equipped with vessels developed by the mother. There is no direct connection between the two sets of blood vessels, therefore, no actual flow of blood from mother to embryo.

However, glucose, oxygen, amino acids, and other materials diffuse from the mother's blood into that of the developing embryo.

The injected chicks received the drug directly from the yolk, so the chick was sure to receive some of the drug. On the other hand, the mammalian embryo does not receive a drug taken into the mother's body unless it will diffuse from the mother's blood into that of the embryo. This means that a drug that greatly deforms a chick embryo may not harm a mammalian embryo simply because it does not reach it. Through extended study of the drug and its mode of action, it can be predicted whether or not this drug will reach the mammalian embryo. A drug that reaches a mammalian embryo should have an effect similar to that which it has on the avian embryo. This especially should be true during the early stages of development.

Further Research. One of the most serious problems of this project was the limited amount of data. The more embryos tested, the more valid the results. This deficiency must be taken into account. For this reason, before continuing much further on the project, the use of a larger incubator is indicated in order that more eggs may be processed simultaneously.

Continued research is planned on the individual components of the drugs already tested. Professional aid will be sought in obtaining appropriate new drugs to test, thus making the original plan or research plausible.

Further investigation is contemplated into the development of the normal embryo to see why certain drugs have specific effects. This would be similar to the work of Stockard (1921), who found that a chemical administered at a certain time in development might have a different effect than if given at another time.

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