The regression coefficients of the percentage of P^{32} on weight and their standard errors were calculated for the first 15 experiments (61 rats), since in the last 4 experiments there was no significant variation in weight. These were —0.09 ± 0.02 before and —0.008 ± 0.02 after correction, the effect of weight thus being almost entirely eliminated by the correction.

In Table I are shown the percentage of nuclei in mitosis and the P^{32} per gram of perfused liver at from 4 hours to 5 days after partial hepatectomy. Mitosis reached a maximum on the first, second, and third days for animals 1, 5, and 16 months old respectively. P^{32} was at a maximum at 0, 1, and 2 days for the same groups. There was an apparent correlation between the time required for reaching maximum mitosis and that for reaching the maximum uptake of P^{32}, the latter anticipating mitosis by 1 day. There was no significant change in mitotic rate during the first 4 hours after the operation.

In another set of experiments with rats weighing 50 g the P^{32} was given subcutaneously 24 hours before the liver was removed for assay. These results are shown in Table II. Maximum mitosis and uptake of P^{32} were reached at 1 day. Whether the P^{32} was administered intravenously or subcutaneously, the uptake of the radioactive element was a much less sensitive index of mitotic function of the liver than was the mitotic count itself. For example, while the uptake of P^{32} varied only a few percent between 0 and 1 a day, the mitotic rate increased by more than 1000%.

In animals which received P^{32} intravenously 1 hour after the partial hepatectomy, a greater concentration of P^{32} was found in the liver than in non-hepatectomized animals, which is probably due simply to the decrease in liver volume. This suggests that the progressive decrease of P^{32} found in the liver between the first and fifth days may be more directly related to the increase in bulk of the liver rather than changes in mitotic activity. However, the data cannot be considered to prove that P^{32} uptake is determined exclusively either by changes in liver volume or rate of mitosis.

Conclusions. 1. The time at which mitosis in regenerating liver reaches a maximum varies with the age of the animal. 2. P^{32} uptake by regenerating liver, 3 hours after intravenous administration of labelled Na_2HPO_4, varies with time after partial hepatectomy. This variation may be related to changes in liver volume or to mitotic activity or both. 3. P^{32} retention 27 hours after subcutaneous administration of labelled Na_2HPO_4, shows little variation with time after operation. 4. The mitotic rate provides a useful index which may be used in assaying the effect of various agents on the reproduction of liver cells.

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On the Use of Sodium Sulfadiazine in Surgery on Amphibian Embryos.

S. R. DETWILER AND C. O. ROBINSON.*

From the Department of Anatomy, College of Physicians and Surgeons, Columbia University, New York City.

One of the greatest difficulties encountered in surgery on amphibian embryos (especially Amblystoma) has been the unpredictable mortality. Particularly in experiments upon the central nervous system, if aseptic procedures are not followed, the death rate may be discouragingly high. Long experience has shown that complete superficial healing, following surgical interruption of the central nervous system, is no guarantee of survival. Embryos, in which the wound surfaces are com-

* Guest Investigator. Medical Officer of the Atlantic Division of Pan American Airways, Inc.

TABLE I. Showing Survival of Operated Amblystoma Embryos When Treated with a 1% Solution of Sodium Sulfadiazine as Compared with Those Kept in Spring Water. The Right Brachial Region of the Spinal Cord Was Removed (in 0.4% NaCl) from Embryos in Stages 20-24 (wound not covered).

Group	Egg clutch	No. of operations	Stage	Time (hr) after operation when transferred to: Sodium sulfadiazine	Spring water	No.	Survival (days after operation) 1	2	3	4	5	6	7	8	Total No.	% survival
A	A	20	20	a 10		10	10	10	10	10	10	10	10	10	10	100
				b	10	10	10	10	3	1	1	1	1	1	1	10
B	B	20	21+	a 3		10	10	10	10	10	10	10	10	10	10	100
				b	3	10	10	10	1	0	0	0	0	0	0	0
C	C	70	22—	a 2		35	35	35	35	35	35	34	34	34	34	97.1
				b	2	35	35	32	0	0	0	0	0	0	0	0
D	D	30	21	a 8		15	15	15	15	15	15	13	12	12	12	80
				b	8	15	15	9	0	0	0	0	0	0	0	0
R	—	50	23-24	a 12		25	25	25	25	25	25	25	24	24	24	96
				b	12	25	25	25	20	12	0	0	0	0	0	0

Group A—All embryos in Aa transferred to spring water on third day after operation.
Group B—All embryos in Ba retained in sodium sulfadiazine for 8 days.
Group C—18 embryos in Ca transferred to spring water on second day. Remainder kept in sodium sulfadiazine for 8 days.
Group D—7 embryos in Da transferred to spring water on third day. Remainder kept in sodium sulfadiazine for 8 days.
Group R—All embryos in Ra kept in sodium sulfadiazine for 8 days.

TABLE II. Showing Delayed Healing of Wounds in Operated Embryos Placed in 1% Solution Sodium Sulfadiazine (Operation described in Table I).

Group	Days after operation	Sodium sulfadiazine: No. of embryos	Healed	Almost healed	Slightly healed	Living	Spring water: No. of embryos	Healed	Almost healed	Slightly healed	Dead
A	2	10	0	2	8		10	4	2	4	0
	3	10	1	4	5		4		2	2	6
	4	10	10	0	0	10	1			1	9
B	2	10	0	7	3		10	0	10	0	
	3	10	5	5	0		1	0	0	1	9
	4	10	10	0	0	10	0				10
C	1	35	0	0	35		35	5	18	12	0
	2	35	0	19	16		32	12	9	11	3
	3 a	17	12	5	0						
	b	18	17	1	0		0				35
	4	35	35	0	0	35	0				35
D	1	15	0	8	7		15	2	13	0	
	2	15	0	6	9		12	10	2		3
	3	15	1	10	4		0				15
	4	15	14	1	0	15	0				15

A—Embryos placed in sodium sulfadiazine 10 hr after operation. Changed to spring water 3 days after operation.
B—Embryos placed in sodium sulfadiazine 3 hr after operation. Retained in sodium sulfadiazine.
C—Embryos placed in sodium sulfadiazine 2 hr after operation. Ca retained in sodium sulfadiazine. Cb transferred to spring water 2 days after operation.
D—Embryos placed in sodium sulfadiazine 8 hr after operation. Retained in sodium sulfadiazine.

pletely closed, frequently break down in regions remote from the site of operation. When disintegration of the embryo takes place it usually occurs 3 to 5 days after the operation.

Experience has shown also that survival varies with different clutches of eggs. Some eggs are presumably more hardy than others. The same type of operation, under identical technical procedures, may (with one egg mass) result in low mortality; in another it may be high. Evidence from long term experience indicates too that viability may vary with eggs from different geographical localities and from year to year.

In 1930, Woerdeman[1] described a method of sterile technic which insured a high percentage of survival in urodele embryos as compared with non-sterile procedures. This method with modifications has been employed by experimental embryologists (Harrison, Schotté, Detwiler, and others) with considerable success in recent years. It involves sterilization of all glassware, operating fluids, and operating instruments. The embryos are transferred successively through 9 to 10 washings in sterile water—using a sterile pipette for each transfer. Whereas this procedure, on the whole, has greatly lowered the mortality, the method cannot be regarded as entirely aseptic in the bacteriological sense, for the embryos are merely washed repeatedly and are probably not rendered entirely free from bacterial invasion into open wounds. Personal experience (Detwiler) over a period of 8 years has shown that this technic, although distinctively advantageous, does not always guarantee the expected results. Usually, however, the markedly greater survival has justified its use despite its laboriousness.

During the spring operating season (1945) we attempted to study restitution of the spinal cord, and subsequent limb function following unilateral excision of the brachial region.† In embryos with uncovered wounds, we encountered almost 100% mortality. Only 3 of 280 operated embryos survived. As a result of this discouraging yield we ventured to study the possibility of employing sulfa drugs. The results have not only been gratifying, but far beyond expectations. After preliminary tests, it was found that young embryos would undergo normal development and growth when kept in a 1% solution of sodium sulfadiazine (Sharp and Dohme) made up in spring water (1000 mg per 100 cc).

The operations reported above, and which resulted in nearly 100% mortality, were repeated. Following the operation, which was done in 0.4 NaCl solution, the embryos were divided into two equal groups. The specimens of one group were placed in individual Syracuse dishes containing a 1% solution of sodium sulfadiazine; those of the other group were placed in individual dishes containing spring water. The intervals in hours between the time of operation and transfer of the embryos to either sodium sulfadiazine or spring water are stated in Table I, which gives the results of 5 series involving 190 embryos.

In order to eliminate any possible modifying factor due to egg selection, records were kept on embryos from individual clutches in 4 of the 5 series (A, B, C, D). Of the 90 embryos kept in spring water, only 1 survived the fourth day, whereas of those treated with sodium sulfadiazine, only 5 out of 95 succumbed and 2 of these were 'unhealthy' appearing embryos when the operation was performed. Embryos transferred from the sodium sulfadiazine solution to spring water 2 to 3 days after the operation survived as well as did those maintained for longer periods in the solution of the drug (Table I). Whereas Table I and Fig. 1 give the survivals for 8 days only, it may be pointed out that the larvæ continued to survive until such time as they were discarded because of curvature or were fixed for microscopic study. Complete data on survival are given in Table I. The significant data are shown graphically in Fig. 1. Observations on the healing of the wounds show that, although drug treatment resulted in a 95% survival in these experiments, it did delay healing. The data are given in Table II. Here it is seen that none of the 70 embryos in the sodium sulfadiazine solution were healed on the second day after

[1]Woerdeman, M. W., *Roux's Arch.*, 1930, **121**, 524.

† The operations were done without sterile precautions, and mostly upon embryos just after complete closure of the neural folds.

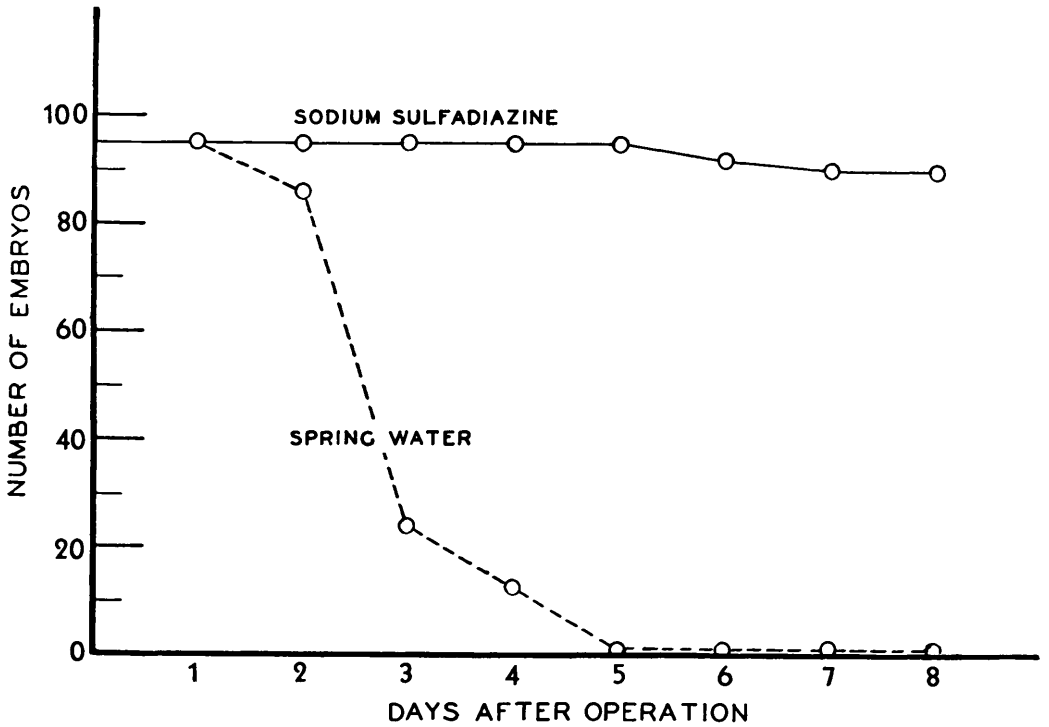

FIG. 1.

A summary of data listed in Table I. The solid line represents survival of embryos transferred after operation to a 1% solution of sodium sulfadiazine; the broken line gives the survival of those kept in spring water.

the operation, whereas 26 of the 70 embryos in spring water had healed over completely. Despite this, however, all but 5 of the embryos in spring water were dead on the third day, whereas 36 of the 70 treated embryos were healed. On the fourth day all sulfa-treated embryos but one were completely healed. It is apparent that death following complete superficial wound healing is due to internal infection and subsequent breakdown. This may be regional involving most frequently the head, or it may involve the whole embryo, with general disintegration.

In one series of unilateral brachial cord excisions in which the wound was covered with ectoderm and underlying mesoderm, the mortality rate was low. Out of 20 embryos kept in spring water, only 5 died (25%). In another group of 20 which were kept in sodium sulfadiazine for 3 days, all embryos survived. Covering the wounds, following extirpation of a portion of the central nervous system, usually insures a higher percentage of survival than in those with uncovered wounds. But even in embryos with covered wounds, the death rate may be high if sterile procedures are not employed, and if the material is not hardy.

The therapeutic value of sodium sulfadiazine is strikingly illustrated by the results obtained by Dr. Ging-Hsi Wang.‡ He made grafts between the medulla and the brachial cord and vice versa (without sterile technical procedure), and obtained only 10 survivals out of nearly 300 operations. After the beneficial effects of the drug were discovered, he repeated the same type of operation upon 24 embryos and obtained 21 survivals. All the more striking are these latter results since the operations were done on embryos which had been kept in the refrigerator for nearly 4 weeks at a temperature of 5°C. Such material typically yields poor results.

‡ Director, Institute of Psychology, Academia Sinica, Chungking, China. Guest investigator under the auspices of the Division of Cultural Cooperation, Department of State, U. S. Government.

Although we have not determined the minimum concentration and length of time necessary to insure survival with the sodium sulfadiazine treatment after operation, our results do show that treatment with a 1% solution for 2 days is adequate (Table I, Group Ca). Furthermore, operated embryos treated for 8-10 days show no deleterious effects of the drug. The developmental rate keeps pace with the controls provided the temperature is the same for both groups. The concentration which we used probably is much stronger than necessary to insure maximum survival. Unfortunately our observations were not made until near the termination of the operating season, and adequate material was not available this year to make further projected studies.

Observations show that even prolonged treatment with the drug has no visible effect upon growth and normal physiological function. This is apparent from a study of two series as follows: Normal embryos in stage 21 were placed in the sodium sulfadiazine solution and maintained there for 4 weeks. Since sulfadiazine gradually crystallized out in dishes containing the embryo, the solution was augmented several times during the first 2 weeks by the addition of freshly made stock solution. During the last 2 weeks, the solution became diluted gradually because of the daily addition of daphnia introduced with a pipette. Throughout this period the animals showed no deviations in development and behavior from those kept in spring water.

In one series (Group 1), 6 embryos were placed in sodium sulfadiazine solution and 6 in spring water. Fifteen days later (just prior to the feeding stage) the 6 treated larvæ averaged 15.8 mm in length; the 6 in spring water averaged 15.3 mm. In another series (Group 2), after 14 days, 6 treated larvæ averaged 14.8 mm, and 6 control embryos averaged 14.5 mm in length. All of these larvæ, whether treated or not, began feeding at the same stage (stage 45-46). At 21 days (7 days after the feeding stage) the treated larvæ in Group 1 averaged 19.3 mm in length; those in spring water averaged 18.6 mm. In Group 2 (6 days after feeding) the average length for both treated and control larvæ was the same (17.6 mm). At the end of 4 weeks, under similar conditions of feeding and temperature, the lengths of the sulfa-treated larvæ in Group 1 averaged 21.6 mm; the spring water controls averaged 22.4 mm. The treated larvæ in Group 2 averaged 22.1 mm; those in spring water averaged 22.1 mm.

Although there are many aspects of the problem which call for further work, nevertheless the present experiments indicate clearly the beneficial use of this drug in surgical procedures on the amphibian embryo.

Summary. The therapeutic effects of sodium sulfadiazine upon Amblystoma embryos, following surgical interruption of the central nervous system, are described. Embryos which may undergo 100% mortality when sterile technic is not employed, will survive when treated for 2 days with a 1% aqueous solution of the drug. There is no evidence of any toxic effect. Treated embryos undergo normal development and at a rate parallel with control embryos kept at the same temperature.

Minimum concentrations of the drug as well as minimum length of treatment necessary to insure survival remain to be determined.