the herpetic inoculation was preceded by shock, 2 (G 137, 138) developed severe encephalomyelitis on the seventh day and were killed when moribund. The third, becoming ill on the eighth day, showed less marked but definite signs of encephalitis and none of myelitis. The 3 animals were autopsied and the presence of typical herpetic lesions noted in the brain and in rabbits G 137, 138, in the spinal cord. Bio-assays of the latter 2 animals revealed virus in the midbrains and spinal cords. Of the 3 rabbits inoculated without the preceding shock, none became ill nor showed herpetic lesions at autopsy 21 days later.

Experiment II. Seven young adult rabbits (2.9-3.5 kg) were inoculated with 1 cc of the supernatant from a 10% suspension of infected mouse brain in Locke's solution. Ten to 15 minutes preceding this inoculation, 4 of the animals (G 151, 152, 153, 154) were given a severe anaphylactoid shock by intravenous injection of 2.75 mg histamine diphosphate. By the seventh day, these animals had all shown signs of herpetic encephalitis. At autopsy widespread lesions were found in the nervous system. Of the 3 animals not shocked previous to the herpetic inoculation (G 148, 149, 150) 2 showed neither signs nor lesions of the disease while one developed a mild encephalitis on the seventh day. At autopsy lesions were found in the midbrain and thalamus of the latter animal.

Discussion and Summary. Intravenous infection of rabbits with neurotropic herpes simplex virus is only occasionally possible according to Le Févre de Arric and Millet,[1] Doerr and Vöctung,[2] and Van Rooyen and Rhodes.[3] This fact is borne out by our control series. The fact that all experimental animals in the series here reported were infected clearly reveals the potentiating effect of histamine shock on susceptibility to the virus. It would seem, from the first animal reported, that the effect is related to a common denominator of histamine and true anaphylactic shock.

2 Doerr, R., and Vöchtung, R., *Rev. gén. Ophthal.*, Paris, 1920, **77**, 372.

3 Van Rooyen, C. E., and Rhodes, A. J., *Virus Diseases of Man*, London, 1940.

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Effects of Sulfonamides on Chick Brain Tissue Cultivated *In vitro*.*

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Although it has become an established practice to apply certain sulfonamide compounds to brain exposed by craniotomy or as a result of war wounds, little is known of the effect of these drugs on brain elements. In an effort to establish a basis for comparison of the toxicity of sulfonamides, brain tissue of the chick was cultivated in plasma containing the following compounds: (1) sulfanilamide, (2) sulfapyridine, (3) sulfathiazole, (4) sulfaguanidine, (5) sulfadiazine, (6) nicotinyl sulfanilamide, (7) sulfapyrazine, (8) succinyl sulfanilamide, (9) succinyl sulfathiazole, (10) sodium sulfapyridine, (11) sodium sulfathiazole, and (12) sodium sulfadiazine.

Methods. In order to avoid the introduction of variables, the procedure of cultivation was reduced to the simplest possible elements. Brain tissue was excised from 8-day old chick embryos and placed in a Petri dish containing Tyrode's solution. The meninges were care-

* The work described in this paper was done under a contract recommended by the Committee of Medical Research between the Office of Scientific Research and Development and the University of California.

fully dissected off and the brain cut into fragments of approximately 0.5 sq. mm. The fragments were quickly transferred for planting to Carrel D-3 flasks containing a medium of chicken plasma.

The plasma was obtained by bleeding roosters from the carotid arteries without anaesthetic. The blood was drawn off into chilled paraffined tubes, then centrifuged for 20 minutes, whereupon the supernatant plasma was pipetted off into unparaffined tubes and stored in the refrigerator.

The method of planting was standardized. Into each Carrel flask was introduced 1.5 cc of plasma. The plasma employed at each initial planting was fresh (under 2 days), and in each instance the remainder of the identical plasma was reserved for patching. Approximately 5 plants were made per Carrel flask and as coagulation of the medium occurred after the introduction of the tissue, the use of embryonic extract was avoided. The flasks were then stoppered and incubated at 38°C. On the average of every second day the cultures were removed from the incubator, washed in Tyrode's solution for a period of 5 to 10 minutes, the fluid decanted, and the culture patched with 0.5 cc of the same plasma used initially. When digestion of plasma proceeded with unusual rapidity, patching was done daily. Explants from subcultures were not done since it was felt that the conditions obtaining in the local application of sulfonamides in the treatment of wounds of the brain could best be met by maintaining the original plant over the prescribed period.

In the experimental cultures, the same technique was employed except that a weighed amount of a sulfonamide was added to the plasma used for planting and patching. All of the sulfonamides employed were weighed out, placed in tubes and sterilized by dry heat in an oven at 140°C for 3 hours. To each tube of the sterilized compound the requisite amount of plasma was added to obtain the concentration desired for both planting and patching, the concentration extending with each drug, from below to somewhat above its saturation point in plasma maintained at 38°C. With each test series, collateral controls, grown in plasma from the same source, were included.

Of the several available methods for measurement of growth in tissue cultures, one similar to that of Ebeling[1] was adopted. Immediately after planting, the cultures were projected by means of a projectoscope modified for this purpose, identified, and the initial plant outlined on paper. On the sixth day, at the conclusion of the experiment, the flask was again projected and the area of extension traced. The surface areas of the tracings of both plant and growth were then measured with a planimeter. From the total area of the plant and its growth, derived by addition of the areas of all cultures comprising a number of a series, the ratio of plant to growth was established. The projection was done at high magnification, 59 diameters, in order to exclude all areas of plasma uninvaded and to furnish a means of outlining the extension of neurones and neuroepithelium. Areas of wandering cells were excluded. After tracing the projected growth on paper, the cultures were viewed microscopically and, where necessary, appropriate modifications were introduced into the traced outline.

From preliminary work it was found unnecessary to make adjustments in pH of the plasma, as a rich growth could be obtained in a test period of 6 days with the routine described above. The factor of pH of the sulfonamides had, however, to be taken into consideration in this study. The first step was to determine the pH of saturated solutions of sulfonamides in plasma at 38°C. This was done by means of a standard Berckman pH meter. The pH of the sulfonamides was found to range from 6.75 to 10.20, most of them between 7.15 and 7.57 (Table I).

The next step was to set up an experiment to test the effect of pH alone on brain cultures. The method of culture was identical to that employed in the foregoing sulfonamide studies. The plants were irrigated twice daily with a solution in the desired range of pH. The solutions were prepared as follows: To "glucosal" (Tyrode's solution without the addition of sodium carbonate) from which

[1] Ebeling, A. H., *J. Exp. Med.*, 1921, **34**, 231.

TABLE I. Data on Growth of Chick Brain Cultures in Plasma to Which Sulfonamides Were Added in Various Concentration. The figures are those obtained 6 days after planting.

Sulfonamide	Approx. solubil. in plasma, mg/cc	pH of sulfonam. at satur. in plasma, 38°C	Range of concentr. of sulfonam. tested, mg/cc	No. cul. grown, incl. controls	Growth ratio of controls	Conc. up to which growth of experimentals was optimal	Corresp. growth ratio	Min. concentr. proving toxic	Corresp. growth ratio
Sulfadiazine	0.4	7.15	0.01- 2.0	283	1: 6.5	2.0	1: 8.3	†	†
Succinyl sulfathiazole	2.0	7.48	0.10- 6.0	229	1: 8.1	2.0	1:15.4	†	†
Sulfapyrazine	1.0	7.70	0.20- 2.0	145	1: 4.0	0.5	1: 5.5	2.0	1:4.3
Sulfapyridine	0.6	7.55	0.10- 1.0	208	1: 4.5	0.3	1: 5.3	1.0	1:4.0
Sulfaguanidine	1.0	7.57	0.20- 2.0	147	1: 3.0	1.0	1: 4.0	2.0	1:2.5
Sulfathiazole	0.7	7.20	0.20- 3.0	175	1: 2.6	0.5	1: 2.8	1.0	1:2.0
Succinyl sulfanilamide	1.0	6.75	0.20- 6.0	136	1:13.7	0.2	1:10.2	0.5	1:5.5
Nicotinyl sulfanilamide	0.8	7.57	0.02- 1.0	234	1: 4.0	0.02	1: 2.1	0.02	1:2.1
Sulfanilamide	9.0	7.50	2.00-12.0	328	1: 2.6	2.0	1: 3.4	4.0	1:1.6
Sodium sulfadiazine	900.0	9.20	0.20- 6.0*	220	1: 5.1	0.5	1: 7.6	6.0	1:2.2
Sodium sulfathiazole	800.0	9.20	0.10- 6.0*	226	1: 5.4	1.0	1: 5.8	3.0	1:2.2
Sodium sulfapyridine	500.0	10.20	0.30- 2.0*	75	1: 5.0	0.3	1: 2.5	0.3	1:2.5

*Owing to the high degree of solubility of these drugs and the unlikelihood of their local use at saturation it was deemed advisable to test these compounds within a range corresponding to the others.

†Cultures grew well at all concentrations tested. Concentrations higher than 2.0 mg/cc could not be tested because crystals of drug obscured visibility.

acid sodium phosphate had been withheld, was added an indicator (phenol red or thymol blue) and then, after sterilization, the solution was adjusted to the desired pH by the addition of the necessary amounts of sterile standard sodium hydroxide solution. In this manner pH concentrations ranging from 5.0 to 9.6 were obtained.

Owing to the buffering power of relatively small amounts of plasma, there was considerable difficulty in maintaining the pH of the cultures to the desired level. This was particularly true of efforts made to reduce the pH of cultures to the acid side even though as much as 1 to 2 liters were employed as the irrigant. Taking this condition of experiment into consideration it may be said that with partial maintenance of the pH at the desired levels the effect of pH on brain cultures was as follows: at pH 5.0, 5.3 and 6.6 there was no growth; at 7.0 a slight outgrowth of neurones as well as moderate neuroglial extension and considerable vacuolization of all cells; at 7.4, and 7.8, excellent growth; at 8.0, moderate to slight growth; at 8.2 to 8.5, slight growth with early vacuolization of cells; and at 9.0 and 9.6 very slight growth with early and severe vacuolization. Thus, the range of pH most favorable to growth of brain in culture was between 7.4 and 7.8. In a parallel series of heart cultures of 8-day-old chick embryos, 297 cultures in all, which were planted in the same flasks as the brain, the growth was also most prolific in this range of pH but there was moderate growth, with evidence of early degeneration, at pH of 6.6. to 7.4 and from 8.0 to 9.6.

From these figures it is apparent that the pH of most of the sulfonamides was well within innocuous limits, and it seems logical to conclude that where the levels of pH were slightly above or slightly below the ideal range the effects were obviated by the buffering power of the plasma. Hence these preliminary experiments seem to warrant the conclusion that the effect exerted by sulfonamides on brain cultures were due to innate properties of the drugs, and not to their pH.

Results. Data on the growth of chick brain cultures in plasma to which sulfonamides were

added are given in Table I. Except for the sodium salts, which are considered separately, the sulfonamides are listed in order of increasing toxicity. Sulfadiazine was the most satisfactory of the group. Cultures grew well at all concentrations tested, even up to five times saturation in plasma. Planimeter measurements suggested that the drug had a stimulating action at higher concentrations. Sulfadiazine was the least soluble of the series (Table I) and its pH in plasma was somewhat below the optimum. Next in order came succinyl sulfathiazole and sulfapyrazine, the growths equaling or somewhat exceeding those of the controls at all concentrations tested. Then came sulfapyridine and sulfaguanidine: cultures containing these drugs grew well up to saturation and somewhat beyond; only at about double the saturation point was the growth less than in the controls. Sulfathiazole, next on the list, when at half saturation had no inhibiting influence on growth, but at saturation and beyond there was early and severe degeneration of nerve cells. Succinyl and nicotinyl sulfanilamide and sulfanilamide itself fall into much the same class inasmuch as they are toxic at approximately half saturation. Of these, succinyl sulfanilamide has a selective effect at concentrations in excess of saturation, producing dissolution of the initially migrating nerve cells and severe vacuolization of neuroglial and wandering cells. Growth of cultures to which sulfanilamide were added was satisfactory up to half saturation; above saturation there was no growth whatever.

The sodium salts, because of their high solubility, were tested over a limited range in the lower concentrations. Judging these compounds at like levels of percentage concentration of saturation, they fall into the following order of increasing toxicity: sodium sulfadiazine, sodium sulfathiazole and sodium sulfapyridine. Cultures containing sodium sulfadiazine and sodium sulfathiazole, respectively, grew as well as the controls up to concentration of approximated 2.0 mg/cc.

Conclusions. Brain of 8-day-old chick embryos cultivated *in vitro* in plasma to which sulfonamides in various concentrations were added, responded differently in accordance with the sulfonamide tested. Cultures containing sulfadiazine and succinyl sulfathiazole grew better than the controls in all concentrations tested. The growths in sulfapyrazine and sulfapyridine equalled those of the controls. The other sulfonamides showed varying degrees of toxicity. Preliminary experiments suggested strongly that the toxicity is to be ascribed to inherent properties of the drugs, not their pH.

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Effect of Digitalization on the Coagulation Time in Man.

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Within recent years a number of communications concerning the influence of digitalis on the coagulability of the blood has appeared. Tanaka[1] found that parenterally administered strophanthin shortens the blood coagulation time of dogs and Werch[2] reported that digifolin exerts a similar effect in rabbits. Macht[3] observed that several of the glycosides shorten the coagulation time of cat plasma to the

1 Tanaka, H., *Okayama-Igakkai-Zasshi*, 1928, **40**, 1826.

2 Werch, S. C., *Quart. Bull. Northwestern U. M. Sch.*, 1943, **17**, 50.

3 Macht, D. I., *Ann. Int. Med.*, 1943, **18**, 772.