

mild shock, respectively. All of the 10 controls showed shock, 2 died, 1 was severely shocked, and the rest showed moderate or mild shock.

Three weeks later some of the survivors of both the experimental and the control group were again challenged without the drug with 0.6 cc of egg white, i.v. All showed shock.

TABLE 1
EFFECTS OF ADMINISTRATION OF 15 GRAINS (.968 Gm) OF
ACETYSALICYLIC ACID, IN DIVIDED DOSES,
ON ANAPHYLACTIC SHOCK

No.	Dosage	Shock	21 days later
931	15 grains in 3 doses	0	..
935	"	0	3
937	"	0	4
939	"	0	1
941	"	0	..
943	"	0	..
945	"	0	..
947	"	0	..
949	"	0	..
953	"	2	..
955	"	1	..
933	None	3	4
934	"	2	4
936	"	1	1
938	"	1	..
940	"	4	..
942	"	2	..
944	"	2	..
946	"	1	..
948	"	2	..
954	"	4	..

To test the effect of acetylsalicylic acid on histamine shock, 11 rabbits weighing 2-2.5 kg were given 5 grains of acetylsalicylic acid, orally, at 3:30 P.M. on March 11, 1947. This dosage was repeated at 9:00 A.M. on March 12 and again at 2:30 P.M. the same day. One hour later, each rabbit was injected intravenously with 2.75 mg of histamine phosphate. Seven of the 11 died (#4 shock), 2 showed severe (#3) shock, and 2 showed moderate (#2) shock.

The animals which were premedicated with acetylsalicylic acid showed striking protection against anaphylactic shock. It may be presumed, on the basis of the literature reviewed above, that this was due to a decrease in the antibodies at the time of the challenging dose. There is nothing to indicate any protective effect against histamine shock, for though unaccompanied by simultaneous controls, the morbidity in the above series was similar to that reported in a previous communication (1). It is interesting to note that the effects of the drug were temporary and that the treated animals showed considerable hypersensitivity 3 weeks later.

The contrast between the results with this drug and those reported earlier (?) for Benadryl is complete. Benadryl gave good protection, in similar experiments, against histamine shock but was without effect on anaphylactic shock. The present drug, acetylsalicylic acid, however, effectively protects against anaphylactic shock but not against histamine. It is, with the other salicyl-

ates, apparently a true antianaphylactic drug in that it interferes with the antigen-antibody reactions to prevent or decrease the untoward results of the challenging dose of antigen.

References

1. CAMPBELL, BERRY, BARONOFKY, IVAN D., and GOOD, ROBERT A. *Proc. Soc. exp. Biol. Med.*, 1947, **64**, 281.
2. COBURN, A. F., and KAPP, E. M. *J. exp. Med.*, 1943, **77**, 173.
3. DERICK, C. L., HITCHCOCK, C. H., and SWIFT, H. F. *J. clin. Invest.*, 1928, **5**, 427.
4. HOMBURGER, F. *Proc. Soc. exp. Biol. Med.*, 1946, **61**, 101.
5. JAGER, B. V. *Proc. Amer. Fed. clin. Res.*, 1947, **93**.
6. RANTZ, L. A., BOISVERT, P. J., and SPINK, W. W. *Science*, 1946, **103**, 352.
7. SULLIVAN, C. J., PARKER, T. W., and HIBBERT, R. W. *Proc. Soc. exp. Biol. Med.*, 1948, **67**, 508.
8. SWIFT, H. F. *J. exp. Med.*, 1922, **36**, 735.

Effects of 2,4-Dichlorophenoxyacetic Acid on Chicks¹

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With the increasing use of 2,4-D as a herbicide it is important to inquire into the possible toxic effects of the chemical on animals. Several investigators have studied the effects of 2,4-D on mammals. The lethal dose for mice, when injected subcutaneously or intravenously, has been determined by Bucher (1) to be 280 mg/kg of body weight. Mitchell and Marth (2) reported that they fed 200 mg of 2,4-D daily to small experimental animals with no ill effects.

TABLE 1
EFFECT OF ALKANOLAMINE OF 2,4-D ON WHITE
ROCK CHICKS

Dosage (mg of acid/kg of body weight)	Increase in weight at end of four weeks (%)
0.00 (control)	456
.28	444
2.80	469
28.00	427
280.00	373

In the present experiment White Rock chicks were used. The alkanolamine of 2,4-D was administered orally through a pipette. In the first experiment, data for which are given in Table 1, one part of the alkanolamine was diluted with 19 parts of water. The dosages recorded are in terms of the acid equivalent. Five chicks (each weighing approximately 50 gm at the beginning) were used in each group. The chicks were weighed and then were given the appropriate dose (Table 1) three times a week on alternate days for a period of four weeks, making a total of 12 doses. As the chicks gained in weight, the

¹ Contribution No. 210 from the Department of Botany and the Rocky Mountain Herbarium, University of Wyoming.

amount administered was adjusted in order to maintain the original dosage. At the end of four weeks they were again weighed. The table shows the percentage increase in weight for the four-week period.

The differences between the control group and those given dosages of 0.28, 2.80, and 28.00 mg/kg were not significant at the 5% level. The difference between the control group and the group given a dosage of 280 mg/kg was barely significant at the 5% level.

Next, experiments were started to determine the lethal dose of the alkanolamine of 2,4-D when diluted 1:9 with water. Each chick of a group of 5 (each averaging 166 gm) was given one dose of 380 mg/kg of body weight. These chicks survived.

Each chick of another group of 5 chicks (average weight, 111 gm) was given a dose of 765 mg/kg of body weight. All of these chicks died. Postmortem examination revealed a fatty degeneration with a pale mottling of the liver, spleen, kidneys, and heart. Hemorrhagic gastroenteritis was also evident.² Hence, for small chicks the lethal dose of diluted 2,4-D is somewhere between 380 and 765 mg/kg.

The fact that a single dose of 765 mg killed, but not a total dose of 3,360 mg administered over a four-week period (280 mg/kg chicks of Table 1), indicates that the alkanolamine of 2,4-D is not a cumulative poison.

The question might be raised as to the possibility of chicks being killed by feeding on plants which had been sprayed with 2,4-D. At a spraying rate of 1 lb of 2,4-D/acre, a chicken weighing 1 kg would have to consume all of the 2,4-D applied on 72 sq ft within a day or two to obtain a lethal dose.

References

1. BUCHER, N. L. R. *Proc. Soc. exp. Biol. Med.*, 1946, **63**, 204.
2. MITCHELL, J. W., and MARTE, P. C. *Bot. Gaz.*, 1944, **106**, 199.

A New Histological Procedure for Whole Tissue Cultures Grown in Plasma

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Standard textbook procedures (2, 3) recommended for fixing and staining whole tissue cultures in plasma coagula have proven unsatisfactory for the following reasons: (1) The dense fixed coagulum presents a nearly impermeable barrier to the stain, thus necessitating prolonged staining periods; (2) the coagulum itself stains diffusely and heavily, preventing adequate contrast between the cells and the medium and necessitating careful and time-consuming decolorizing and developing procedures; (3) the resulting preparation, when mounted, is thick, has a tendency to form air bubbles, does not

dry readily, and is generally inadequate for microscopic study. Tompkins, Cunningham, and Kirk (4) recently attempted to improve results by washing cultures in a salt solution for 4 hrs at 7° C to remove soluble protein before fixation, but still had to resort to prolonged staining (1 hr in Delafield's hematoxylin) followed by several hours of washing. Earle (1) has devised complicated fixing, staining, and mounting procedures, too elaborate to warrant evaluation here.

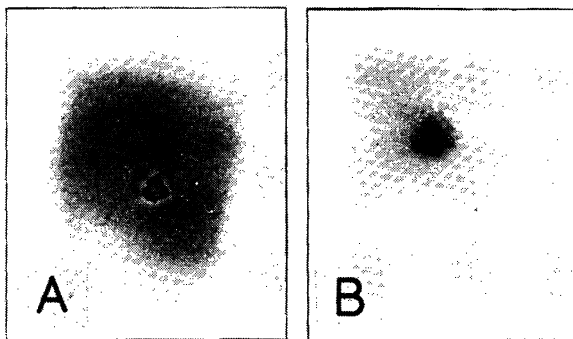


FIG. 1. Gross appearance of stained tissue cultures: A, plasma undried; B, plasma dried ($\times 1.5$).

We have found that most of the disadvantages of the plasma coagulum can be eliminated by drying the whole tissue culture preparation after fixation. The procedure is as follows: The culture in its coagulum on a cover slip is fixed overnight in 3-4% formol containing 0.5% acetic acid or in Carnoy's fluid for 1 hr. In general, acetic-alcohol fixations give a more granular and more opaque dried specimen than acetic-formol fixations. The cover slip is then washed thoroughly in running water (after acetic-formol) or in descending strengths of alcohol, and water (after Carnoy's). After a final rinse in distilled water the cover slips are laid flat on a glass surface protected from dust (Petri dishes or staining dishes can be used) and allowed to dry slowly and thoroughly in air. If a preparation of a tube or a flask culture in plasma is desired, the portion should be fixed and washed *in situ*, pried loose and placed, like a paraffin section, in a dish of water, where it can be manipulated with a dissecting needle onto a slide or cover slip previously coated with a thin film of Mayer's albumin, and dried as above. Cultures grown in a fluid medium should not be dried. The dried cultures may be stained with any one of the hematoxylin for from 5 to 15 min, depending on the strength of the stain. Weigert's iron-hematoxylin has been found very satisfactory. The staining is simple to control after a preliminary trial, and prolonged washing to develop the color is not necessary. Once stained, the cultures should not be allowed to dry again. Following this, the cover slips are passed through the alcohols in the usual manner, cleared in xylene, and mounted. If a Leishman or Giemsa stain is used, acetone and acetone-xylene dehydrations should be employed instead of the alcohols. The dehydration and clearing procedures take considerably less time with dried cultures, since each

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