observations covered a period of 42 days. My records showed the disappearance time of *Spirochaeta pallida* under the influence of penicillin, as well as the healing period required for the testicular lesions. It is well known that normal testicles following antisyphilitic therapy do not constitute reliable evidence of the animal's cure. An additional period of 3 to 4 months' observation after the testicles become normal again is necessary to ascertain the fact of cure. At that time the popliteal lymph nodes are removed from the rabbits and tested for spirochetes by means of their intratesticular transfer into a healthy rabbit, which in turn is observed for 3 months to note evidence of testicular infection.

In this paper I am offering the final results obtained by removing the popliteal lymph nodes 100 to 115 days after the testicles of penicillin-treated rabbits became normal. As stated in my original paper,[4] one syphilitic rabbit received intramuscularly 2,500 Oxford units in aqueous solution, twice a day for 8 consecutive days—a total of 40,000 Oxford units per kilogram body weight. The popliteal lymph node transfer test proved negative and thus indicated cure. The other two syphilitic rabbits were each treated by intramuscular injections of 5,000 Oxford units of penicillin in oil suspension, once a day for 8 consecutive days—again a total of 40,000 units per kilogram body weight. The popliteal lymph node transfers also gave negative results or cure.

Additional experiments, conducted subsequent to our publication, consisted of the treatment with penicillin sodium of 3 syphilitic rabbits. One was given 3,300 units of penicillin sodium in aqueous solution three times daily, *i.e.*, 9,900 units per kilogram body weight per day, for 8 consecutive days. The total penicillin administered in this case comprised 79,200 units (about twice the amount given to the first animal treated with aqueous solution, as described in the previous publication). The result of the popliteal lymph node transfer was negative, indicating cure. Although in both cases cure resulted (negative popliteal lymph nodes), the record shows that the lesions in the animal treated with the higher dosage were freed of spirochetes and healed in a shorter time.

Two more syphilitic rabbits received twice daily 5,000 units per kilogram body weight, of penicillin sodium in oil suspension, *i.e.*, 10,000 units per day for 8 days consecutively. Each animal was given a total of 80,000 units. The syphilitic animals were cured (negative popliteal lymph nodes). Again with the double dose the curative effect was more rapid. The spirochetes disappeared more quickly from the testicular lesions, and the lesions returned to normal sooner than with the smaller dosage.

From my experiments, one may infer that syphilitic rabbits which received 40,000 Oxford units per kilogram body weight in a period of 8 days, whether in aqueous solution or in oil suspension, were cured by every accepted standard of cure for rabbit syphilis. Translated into a treatment schedule for a patient weighing 60 kilograms, the total dose for the patient should be 2,400,000 units. One ought to keep in mind, moreover, that the animals getting twice the dosage used in the original experiments, namely, a total of 80,000 units per kilogram body weight, were not only cured but were cleared of lesions faster. A somewhat more rapid curative effect was noted with penicillin in oil suspension. Since the double dose of penicillin in oil suspension was 80,000 per kilogram body weight, it suggested that total treatment for a patient weighing 60 kilograms should be 4,800,000 units.

GEORGE W. RAIZISS

NON-TOXICITY OF DDT ON CELLS IN CULTURES

IN an attempt to find a standard cell system for studying the effects of DDT (2,2 bis (p-chlorophenyl)-1,1,1-trichloroethane) a number of experiments were performed with cultured tissues. The negative results obtained are presented here briefly.

HANGING DROP CULTURES

A small drop of a saturated alcoholic solution of DDT was allowed to dry on a coverglass. Hanging drop cultures of heart and intestine from 7- to 8-day chick embryos and brain and spleen from a 1-day rat were set up in different combinations of Locke solution, chicken plasma, chick embryo extract and human placental serum, so as to include the dry DDT which remained stuck to the coverglass. The cytology and migration of fibroblasts, entoderm and macrophages were not appreciably different from the control cultures without the DDT during a period of 3 days to one week. Fibroblast mitoses were about as in the controls.

A similar series of cultures of the intestine from 8-day chick embryos and brain and spleen from a 1-day rat, in which saturated DDT in acetone was dried on the coverglass before the cultures were made, were set up in different media. There were no appreciable differences between their outgrowths and those in control cultures. Living fibroblasts as they moved about in the cultures sometimes touched or even migrated over DDT crystals without any appreciable injury to themselves during a period of several days.

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[1] From the Dermatological Research Laboratories, Philadelphia, Division of Abbott Laboratories, North Chicago, Illinois.

[2] The author wishes to acknowledge with thanks the cooperation of Dr. Herman Beerman, University of Pennsylvania.

[3] Dr. Raiziss died on July 16.

[4] George W. Raiziss, SCIENCE, 100: 412, November 3, 1944.

After drying on the coverglass, the DDT is only slightly soluble in water, much less than 1 part to 1,000,000 parts. Its solubility in the culture medium at body temperature is unknown but presumably the medium became saturated in the course of a few hours at 98° F.

Other combinations were tried, such as adding DDT-acetone solution to the Locke solution, and adding small fragments of cockroach chitin to some drops.

Under the conditions of these experiments the DDT had no appreciable effect on cytological characteristics of fibroblasts, macrophages and entodermal cells even when such cells were in actual contact with DDT crystals.

Prolonged Exposure of Cells to a Weak Solution of DDT in Roller Tube Cultures

Four series consisting of 15 roller tube cultures with heart, liver, kidney, stomach, intestine, skeletal muscle, brain and spinal cord in each tube were set up in a standard medium. The clot consisted of 1 drop of chicken plasma plus 1 drop of chick embryo extract. After clotting, a supernatant fluid (7 drops Locke solution plus 5 drops human placental serum plus 1 drop of chick embryo extract) was added.

Enough crystals of DDT were then added to all but the control tubes to get a saturated solution with many crystals left over. The crystals floated around in the medium and frequently landed on the migratory zone. The supernatant was changed every 2 or 3 days and fresh crystals added each time.

The tissues were cultured for 10 to 21 days. The outgrowths usually had many mitotic fibroblasts and more or less abundant outgrowths of fibroblasts, macrophages, entoderm, liver cells, kidney epithelium, nerve fibers and muscle fibers.

They were like the controls and there was no indication that the weak solution of DDT or solid particles of it had any effect on the cells.

Short Exposure of Cells to Concentrated DDT Emulsion in Roller Tube Cultures

In an attempt to circumvent questions arising from the low solubility and questionable penetration from solid or colloidal DDT, a quick-killing emulsion was tried. This emulsion contained 1 per cent. DDT, 9 per cent. olive oil, 1 per cent. gum arabic and 90 per cent. saline. Intracardiac injections of ½ cc of the 1 per cent. DDT emulsion produced symptoms in a few minutes and killed mice in 45 minutes to 76 hours. This emulsion was not sterile, and the contamination it introduced into the tube cultures usually forced termination to explants at the end of 24 hours. However, since the emulsion can kill an intact animal in a few hours, it seems logical that if there were any visible effect on cultured cells it should be visible within this period.

A tube of 9-day chick embryo tissues (heart, kidney, stomach, intestine, liver, muscle) was set up in the standard medium. One drop of the DDT emulsion was added; this gives a relatively high concentration, approximately 0.06 per cent. of DDT. At twenty-four hours there were outgrowths of fibroblasts, macrophages, entoderm, liver cells and kidney epithelium. These cells were in good condition. The next day the infection had increased and the tube was discarded.

To two 43-hour control tube cultures that had good outgrowths of nerve fibers, entoderm, fibroblasts, macrophages and kidney epithelium and fibroblast mitoses, one drop of the 1 per cent. DDT emulsion was added. Four and a half hours later the cells, including the nerve fibers, were in good condition and there were fibroblast mitoses. The next day the tubes had muscle fibers, nerve fibers, fibroblasts, macrophages, entoderm and kidney epithelium, all in good condition, and fibroblast mitoses. A day later the badly infected tubes were discarded.

A tube containing a number of liver explants from a 9-day chick embryo was set up in the usual medium. On the fourth day most of the explants showed outgrowths of liver cells, macrophages and fibroblasts. Mitoses of the latter were common. One drop of DDT emulsion was added to this culture. The next day the liver cells, macrophages and fibroblasts seemed to be in good condition and mitoses of the latter were common. The DDT had not produced any noticeable harm to the liver cells. All the cells were still in good condition the next day.

No DDT crystals were seen in any of the tubes, the emulsion appeared to dilute satisfactorily in the culture media used.

In some respects it may seem peculiar that we were unable to affect the growth of various cells in culture when it is easy to kill mammals by the injection of emulsions or application of solutions of DDT. We do not know the explanation of this difference between intact animals and their cells *in vitro*. Any of several factors might be involved. Cultured cells are a vastly simpler system, not dependent on the complex integration of an intact animal. Cultured cells are also supplied at all times with excess of nutrients and oxygen and with full opportunity to dispose of metabolites.

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