EFFECTS OF COLCHICINE INJECTED IN FEMALE DROSOPHILA.

By: GYULA FÁBIÁN.

(From the Hungarian Biological Research Institute, Tihany, Lake Balaton.)

With 4 Figures and 1 Table in the text.

(Received for publication 30th November 1946.)

In the following experiments I investigated the effect of different concentrations of injected colchicine on adult female Drosophilas and on their eggs when laid.

GELEI and CSIK (1940) established that when the Drosophila's food contains $1:10^3$, $1:10^5$, $1:2.10^5$ diluted colchicine, the flies die after a couple of days without offspring. The animals can survive the $1:3.10^5$ and $1:4.10^5$ dilutions, but with a smaller number of progeny than in the control cultures. HADORN (1946) found on dissecting the gonads of Drosophila larvae that one can treat them in vitro with colchicine and so transplant the gonads, the chemical agent causing a typical injury to them.

My present effort was to see if a similar effect could be produced in the young adult flies with injections of colchicine.

TECHNIC.

For the injection I employed Beadle—Ephrussi's standard method. I diluted the colchicine from a 1/1000th% basic fluid in sterile Ringer solution. I used the following 3 dilutions for the experiment: $1:10^5$, $1:2.10^5$ and $1:3.10^5$. For the injections I employed a 1 cm^3 "Tuberculin" syringe provided with a regulating screw-wheel. The wheel was divided into 36 degrees. A whole revolution of the wheel, i. e., 36°, ejected 0.01 cm^3 of the fluid. To fill the abdomen of a young female Drosophila an average turn of 2° was required. In any case the insects themselves regulated the amount of the fluid, because the membrane between the segments of the abdomen admitted no more liquid once it was full, so that if animals of one size are used the same amount of liquid can always be employed. Thus I can give approximate data on the amounts

of the solutions and the weight of the colchicine, namely, that one example can take approximately 0.00055 cm^3 fluid, and the weight of the colchicine so injected diluted to $1:10^5$ is 1/10th microgram; to $1:2.10^5$ is 1/20th microgram, and to $1:3.10^5$ is 1/30th microgram.

In each series 10—10 examples were injected at exactly the same time, one series when they were 1 hour old, one when 10 hours old, and one when 24 hours old. One series 1 hour of age was taken as control and injected with pure Ringer solution. I registered the production of eggs every 24 hours for 5—6 days.

From the deformed eggs I made histological slices, fixed in Carnoy, Delafield staining for 1 minute.

THE EXPERIMENTS.

The injection of the pure Ringer solution had no effect, either on the adult control examples or on their eggs. Under sterile treatment the perforation soon healed and the swelling of the abdomen disappeared in half a day, after which each example continued its normal existence.

The colchicine, according to the different concentrations, had different effects, and the differences in the ages of the examples had their influence too. The data thereon are given in the following Table.

a.) *Mortality.* Of the examples one hour old receiving colchicine in a concentration of $1:1.10^5$, 90% died. Of the 22 treated females only 2 remained alive at the end of 72 hours. Of the examples 1 hour old receiving the $1:2.10^5$ solution, not one died; of the 10 hour old series 3 examples died, of the 24 hour old, one.

b.) *Sterility.* The colchicine injected rendered the females either permanently or temporarily sterile. The permanent sterility was caused by the $1:1.10^5$ concentration. The 2 examples above mentioned which survived the strongest solution laid no eggs at all. Of the one-hour examples given the milder solution I observed that 2 females remained permanently sterile. In the case of those temporarily sterile I remarked that they did not begin to lay during the 48th hour of life but some 2—3 days later. Of the examples receiving the weaker solution when 1 hour old, among the 10 females 2 delayed laying by 3 days, 2 by 2 days, and 1 by 1 day. Under the same concentration the examples treated later in life were more resistant. Among the 10 females when 10 hours old, 3 delayed 1 day in laying, of those treated when 24 hours old, 8 delayed 1 day. Of those receiving the weakest solution ($1:3.10^5$)

conc.	age of fem. injected.	number of fem. treated.	mortality	permanently sterile	temporarily sterile	deformed eggs in percent	daily production of eggs of 10 fem.
5^{-1}:1.10	1 h	22	20	2	—	—	—
5^{-1}:2.10	1 h	10	—	2	5	100.0	11
						20.0	20
						1.2	73
						3.4	88
						0.0	159
5^{-1}:2.10	10 h	10	3	—	3	65.1	8
						10.6	43
						0.0	75
						0.0	131
						0.0	144
5^{-1}:2.10	24 h	10	1	—	8	0.0	3
						0.0	146
						0.0	143
5^{-1}:3.10	1 h	10	—	—	—	90.5	95
						15.2	327
						0.0	310
5^{-1}:3.10	10 h	9	—	—	—	44.8	98
						20.2	232
						1.9	351
						0.0	260
5^{-1}:3.10	24 h	10	—	—	—	0.0	88
						0.0	434
						0.0	428
Control.	1 h	10	—	—	—	0.0	112
						0.0	327
						0.0	265
						0.0	300

only 3 of the group treated when 10 hours old delayed 1 day in laying, all the others of the 2 groups began to lay their eggs at the normal time on the 2nd day.

In respect to mortality and sterility the general observation can be made that the animals show individual variations in their reactions to the colchicine. But, roughly speaking, it is clear that the mortality and sterility depend in direct ratio to the concentration of the colchicine. The number of animals investigated was not sufficient to establish to what extent the age of the animal influences the effect of the colchicine. Comparing the effect of colchicine as given by injections with treatment in vitro on the one hand and direct feeding of colchicine on the other, it can be seen that it stands somewhere between the two.

c.) *Number of eggs laid.* The average daily egg production of 1 female is given, as evaluated from the average daily production of 10. The number of eggs laid the first 3 days was indicative of the intensity of egg-production. The $1:2.10^5$ concentration gave a small number but increasing slightly according to the advance in age. Under the effect of the $1:3.10^5$ concentration the behaviour was scarcely different from that of the normal controls (Fig. 1).

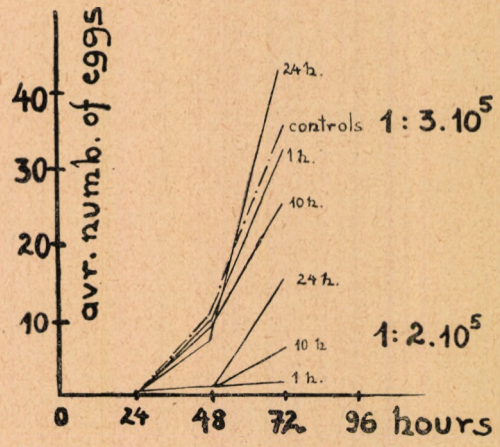

Fig. 1. The average daily egg-production of a treated female in the groups of $1:2.10^5$ and $1:3.10^5$. The average production of the control is represented by the line -.-.-.-.

d.) *Deformed eggs.* The most typical effect of the colchicine was seen in the eggs. Depending on the concentration and age, a definitely determined percentage of misshapen eggs appeared, while not a single deformed egg was present among those laid by the controls (1004 eggs). The shape of the deformed eggs was typical of each given concentration.

The consequence of the $1:2.10^5$ solution was that the 2 long spongy horns typical of the normal egg disappeared entirely and the shape of

the egg lengthened (62 cases). After the 1:3.10^5 solution the long horns also disappeared but a small protusion remained on the dorsal side of the egg (Fig. 2.). (241 cases.)

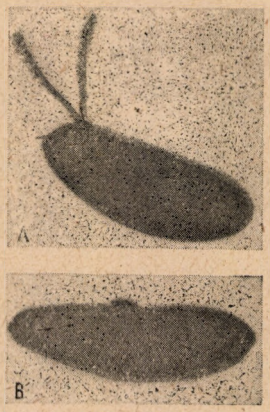

Fig. 2. The normal (A) and the deformed (B) eggs after treating with colchicine (1:3.10^5).

I kept the deformed eggs at a temperature of 25° C to see whether they were capable of producing larvae or not. This did not occur, in a single case.

From the histological examination it appears that the tumour-like protrusion results from an accumulation of chorion cells. In substance and structure it is the same as in the horn of the normal egg, though it is only one, not a pair, and considerably less in number of cells. These empty chorion cells are larger at the base of the protrusion, while those at the top are smaller. The interior of the egg suffered injury in that the differentiation of the tissues stopped.

The percentage of deformed eggs in relation to colchicine concentration and age can be seen in the two following *graphs,* both of them typical in that the older animals better resist the treatment, and with stronger concentration the injury is greater. Further characteristics are that the quite young, 1 hour old, animals react very actively to the colchicine, but the effect quickly wears off. The examples treated when 10 hours old reacted less actively but the effect wore off more slowly.

It appears from these investigations that with the injection of colchicine into the Drosophila organism a functional disturbance is caused which can be said to resemble the effect of colchicine when administered in their standard food. The effect of colchicine when

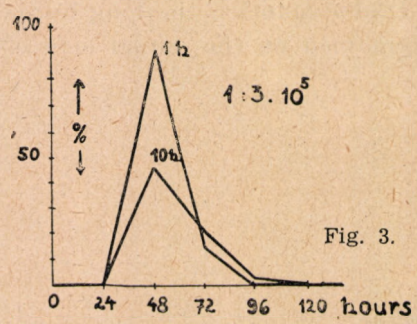

Fig. 3.

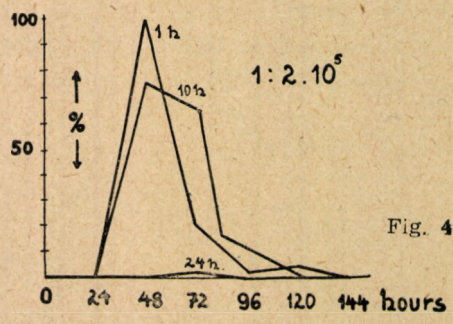

Fig. 4.

Fig. 3. The percentage-axis of deformed eggs after treating the 1 hour and 10 hour old animals with colchicine ($1:3.10^5$).

Fig. 4. The same as Fig. 3. but in the case of a stronger ($1:2.10^5$) colchicine solution.

injected produces 2 factors which cause a reduction in progeny: 1.) the mothers become completely or temporarily sterile; 2.) deformed, and so unproductive, eggs are present to a certain degree. But while the effect of injected colchicine wears off after a few days, the colchicine administered to the Drosophila in food, if not in lethal concentration, causes a permanent though lower equilibrium in the number of progeny, which has been established by GELEI and CSIK in respect to certain degrees of concentration. It is probable that there do not exist in the alimentary system of the Drosophila the sort of enzymes which can destroy the colchicine.

On the other hand, comparing our investigations with those of HADORN, it appears that the ovarium of the newly born females until they reach the age of 24 hours is as sensitive as that of the larva. The characteristic and typical deformation of the eggs indicates the same effect of the colchicine as in the larval stage and in the early adult life.

SUMMARY.

Injections of $1:1.10^5$, $1:2.10^5$ and $1:3.10^5$ concentrations of colchicine in female Drosophila imagos aged 1 hour, 10 hours and 24 hours caused injury in respect to viability and fertility. Moreover, typical injury-effects were to be seen in the eggs when laid, depending on the age when treated and the degree of concentration of the colchicine.

Grateful acknowledgment is made to Mrs. J. THOMPSON VASS for the English translation.

REFERENCES.

GELEI, G. and Csik, L. (1940). *Biol. Zbl.* **60.** 275.
HADORN, E. (1946). *Rev. Suisse de Zool.* 53. 486.