

Vein	Stage of development	Total number of explants*	Number of contractile explants*	Frequency of the contractions at 38-5°C (minimum and maximum values)
Omphalomesenteric vein	14 days incubation	33 (7)	11 (5)	3-12
	15 days incubation	29 (9)	9 (3)	3-13
	16 days incubation	19 (4)	11 (3)	3-20
	17 days incubation	12 (2)	7 (2)	2-10
	18 days incubation	7 (2)	5 (2)	3-18
	19 days incubation	13 (4)	7 (4)	1-14
	20 days incubation	11 (4)	4 (4)	5-13
	Hatching	4 (2)	3 (2)	7-19
	1-5 days after hatching	11 (6)	6 (4)	5-17
	16 days incubation	12 (4)	1	5
Mesenteric vein	17 days incubation	7 (2)	1	6
	19 days incubation	11 (4)	1	4
	20 days incubation	15 (4)	9 (4)	14-16
	1-5 days after hatching	25 (5)	12 (4)	7-15
	17 days incubation	1	1	10
Right portal vein	18 days incubation	2 (2)	2 (2)	6- 8
	20 days incubation	4 (4)	1	6
	1-5 days after hatching	7 (7)	7 (7)	6-11
Left portal vein	18 days incubation	3 (3)	1	8
	20 days incubation	4 (4)	1	12
	Hatching	1	1	7
Umbilical vein (abdominal portion)	1-5 days after hatching	7 (7)	7 (7)	9-18
	16 days incubation	19 (4)	2 (1)	4
	17 days incubation	10 (2)	5 (2)	5- 7
	18 days incubation	7 (2)	2 (1)	2- 6
	19 days incubation	19 (4)	6 (3)	5- 6
	20 days incubation	26 (5)	21 (5)	2-14
	Hatching	1	1	10
	1-5 days after hatching	32 (6)	17 (6)	7-14

* The figure between brackets refers to the number of chick embryos or chickens from which the explants were derived.

of the relations between the muscle cells and the walls of the umbilical vein is now being made.

The results reported above concerning the presence of a spontaneous peristaltic activity in the veins of the portal area and in the umbilical vein of chick embryos and newly hatched chickens, confirm on the one hand the findings which have been obtained with a physiological method by RONCATO on the portal and superior mesenteric veins of adult dogs, and on the other hand support the hypothesis of an active participation of the venous walls in the blood propulsion in those areas: this hypothesis has already been advanced by RONCATO for the veins studied by himself and by other authors (RENAUT¹, DUBREUIL and LACOSTE², BUCCIANTE³) for those veins, especially in man, which are in an unfavourable condition as regards the venous return because of the force of gravity and contain a more or less conspicuous amount of muscular elements in their walls.

Further researches are in progress in order to ascertain whether, in other segments of the venous system of the chick, especially in those in which the blood is flowing, in the fully developed animal, against the pull of gravity, a contractile activity *in vitro* can be demonstrated already during the embryonic life or in the first periods after hatching.

G. ATTARDI and D. ATTARDI-GANDINI

Institute of Histology and General Embryology, University of Padua, June 19, 1954.

¹ J. RENAUT, *Traité d'Histologie pratique* (Rueff et Cie, Paris, 1888).
² G. DUBREUIL and A. LACOSTE, *Ann. Anat. path.* 8, 988 (1931).
³ L. BUCCIANTE, *Arch. it. Med. sper.* 7, 361 (1940); *Medicina e Biologia* 2, 35 (1943).

Résumé

Les veines du système porte et la veine ombilicale gauche d'embryons de poulet dans la dernière semaine d'incubation et de poussins à l'éclosion ont été cultivées *in vitro*. Les auteurs y ont démontré la présence de contractions péristaltiques spontanées. Ils pensent que cette activité contractile rythmique favorise *in vivo* la circulation dans ces territoires veineux.

PRO EXPERIMENTIS

A Method for the Destruction *in situ* of Gonads of *Drosophila* larvae

Introduction.—In connection with experiments involving ovary transplantations between different stocks of *Drosophila* larvae, the need had arisen for removing or destroying a larval gonad with as little damage as possible, so that further operations, such as the implantation of a gonad from a different stock, might be successful on the same larva. It had been proved possible to remove mechanically one ovary with a good survival rate¹, but very difficult to remove one ovary and then to implant another one or to remove both ovaries with success, because of the great loss of body fluid and damage to the cuticle and inner organs.

To overcome this difficulty, a method for destroying a larval gonad *in situ*, with a minimum of damage or loss of body fluid, has been developed and tested.

¹ E. M. PANTELOURIS (in press).

Its principle is as follows: a microelectrode, insulated except at its very tip, is inserted through the larval cuticle so that its tip touches the gonad; a current of sufficient intensity to destroy the gonad is then passed. To ensure localisation of the effect, a comparatively broad metal plate is used for the "indifferent" electrode; thus, current spreads out from the microelectrode tip on to the broad plate and, therefore, current density diminishes with distance from the tip, falling off to a level harmless to other tissues. To avoid electrolytic by-effects, alternating current of sufficiently high frequency is used.

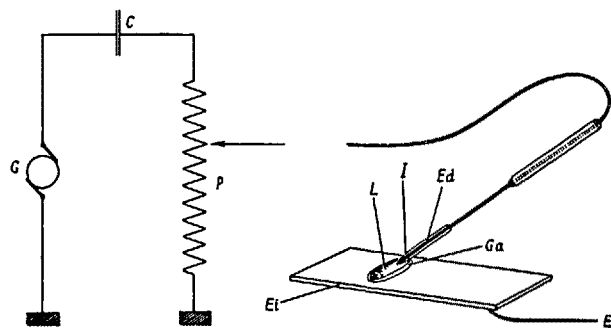


Fig. 1.—Apparatus for the destruction of gonads *in situ*. *G* = source of current. *C* = Condenser. *P* = Potentiometer. *Ed* = Tungsten microelectrode. *I* = Insulating polythene sleeve round the microelectrode. *Ei* = Metal operating plate acting as the "indifferent" electrode. *L* = Larva fixed on the metal plate by means of *Ga* = a film of syrup of gum arabic in RINGER solution.

Apparatus.—Figure 1 shows diagrammatically the apparatus constructed. A high frequency tester¹ was used as source (*G*) of current, giving a nonsinusoidal alternating current of a frequency of about 20 kcs/s. The larva *L* is placed on to the metal plate ("indifferent" electrode *Ei*) in a drop of electrolyte solution *Ga*. The voltage between *Ed* (microelectrode) and *Ei* is regulated by means of the potentiometer *P*. A condenser *C* is inserted in the circuit in order to eliminate any direct current from the source. The microelectrode is fastened on to an insulated handle which can be moved by a micromanipulator (SINGER's type) to facilitate manipulation and ensure exact localisation.

Success depends largely on the microelectrode. This must be, together with its insulating layer, very fine so as to leave as small a wound as possible, but also rigid enough to pierce the larval cuticle. Steel, wood's metal, platinum and tungsten were tried for the microelectrode, and glass, colophonium, collodion and polythene for the insulating layer. Tungsten with polythene insulation was found to be the best.

The microelectrode is constructed as follows: Tungsten wire of 0.075 mm diameter is provided with a fine tip by dipping into boiling NaNO_3 . A small ball of polythene in size of a cherry-stone is warmed until it becomes viscous and the tip of the electrode is dipped into it and withdrawn. A sleeve of polythene, continued in a stalk and a ball of the same material at the end, remains attached to the electrode. The polythene sleeve is still too thick. It can be made thinner on the microforge (DE FONBRUNE's type). The wire with its polythene sleeve is mounted vertically on to the microforge. The platinum heating filament of the forge is laid around it in a spiral (Fig. 2) without, of course, touching the polythene. Then the filament is heated carefully until the polythene around the wire becomes viscous. It becomes stretched by the weight of the adherent ball and this diminishes

the thickness of the sleeve; when this is thin enough, the polythene stalk is heated near the tip of the electrode until it breaks off, leaving a fine insulating layer around the electrode except at the very tip. The insulation of the electrode is tested by connecting it to the circuit, dipping it into a drop of an electrolyte solution and passing current through it. Gas bubbles, observed under the binocular, must only come off the tip and not the sides of the electrode.

Technique.—The larvae to be operated are sterilized by immersion in 70% alcohol for a short time, are then etherized, and placed on the indifferent metal-plate electrode. It was necessary to devise a means for keeping the larvae fixed, for otherwise they contract violently when the current is passed, although they are etherized. When this happens, the tip of the microelectrode is displaced and does not touch the gonad. It was found convenient to cover the larva with a layer of thick syrup of gum arabic dissolved in RINGER's. This syrup dries and forms a transparent and hard shell over the larva. By its rigidity it immobilises the larva and also facilitates the insertion of the microelectrode. The microelectrode is then inserted, under the binocular and with the help of the micromanipulator, through the gum shell and the cuticle, and its tip is brought to touch the gonad which is visible through the body wall. The current is switched on for a few seconds and then the electrode is withdrawn. The gum shell is washed away with RINGER's, and then the larvae are transferred on to sterile moist filter paper. After a short time the wound has healed and the larvae are transferred to food vials. The most suitable voltage between the electrodes is chosen empirically.

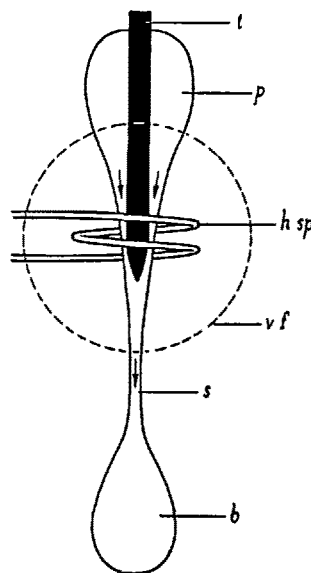


Fig. 2.—Construction of the tungsten microelectrode. *t* = Tungsten wire. *p* = Polythene layer around the wire, continuing through a stalk, *s*, to a ball, *b*, of the same material. *h.sp* = Heating platinum spiral of the microforge. *v.f.* = visual field of the horizontal micro-scope of the microforge.

Results.—The effectiveness of the operation was investigated by dissecting the flies hatched from the operated larvae. All operated larvae were at third instar stage.

In females, the ovary on the operated side was missing or more or less reduced in size. In the males the testis on the operated side was either missing or reduced in size and also abnormal in shape and lacking its normal yellow

¹ Model T 1, made by W. Edwards & Co. (London) Ltd.

pigmentation. In one of the latter cases, as well as in a case where the testis on the operated side did not show any visible damage, microscopical investigation showed no normal sperms, although the unoperated testis of the same animals showed normal sperms.

The operation is, generally, more successful in males than in females, possibly due to the large size of the male gonad which allows better localisation of the treatment. Further, it is possible to push the tip of the electrode into the testis, thus adding mechanical damage to the effect of the current.

Effect of operation on third instar larvae of Drosophila melanogaster. 142 adults hatched out of a total of 215 larvae treated. The percentage of hatching adults was 56% for the males and 66% for the females for the whole series of tests, but it was much higher for the last experiments in the series.

Adults dissected	Sex	Gonad missing or greatly reduced	Gonad only slightly reduced	Gonad not visibly damaged	Doubtful
94	F	20	23	40	11
48	M	15	13	15	5

The results of the tests are shown in the table which summarises all experiments except some preliminary

ones. Of course, success depends largely on the experimenter's skill and the refinement of the method. Therefore the summarised experiments give no real indication of the possible survival rate, which indeed increased from experiment to experiment. In the last and best experiment 15 flies hatched of 20 operated female larvae, showing ovary destructions in 13 cases.

Acknowledgments.—This work was carried out at the Institute of Animal Genetics, West Mains Road, Edinburgh, Scotland. The authors are greatly indebted to the Director, Professor C. H. WADINGTON, F. R. S., for providing facilities and for his interest and encouragement during the course of the work. One of us (D.P.) wishes to thank the Deutsche Forschungsgemeinschaft for a travel grant.

E. M. PANTELOURIS and D. POGGENDORF

Institute of Animal Genetics, West Mains Road, Edinburgh, Scotland, and Entwicklungsphysiologisches Institut der Universität Köln, Germany, August 20, 1954.

Zusammenfassung

An männlichen und weiblichen *Drosophilalarven* im dritten Larvenstadium lassen sich die Gonaden durch Hindurchleiten eines hochfrequenten Wechselstroms (Frequenz 20000 Hz, Stromstärke empirisch) ohne Schädigung anderer Organe *in situ* zerstören. Die Methode ist bei Männchen erfolgreicher als bei Weibchen. Die Versuchsanordnung und die Herstellung einer geeigneten Mikroelektrode werden beschrieben.

Informations - Informationen - Informazioni - Notes

STUDIORUM PROGRESSUS

Die tektonische Metamorphose der Kohle

Eine Überschau neuerer Arbeiten

Von W. E. PETRASCHECK jun., Leoben¹

Die Ursachen der Reifung der Kohle sind mannigfach. Druck und Temperatur sind die Hauptfaktoren, aber auch Unterschiede des pflanzlichen Ausgangsmaterials, geochemisches Bildungsmilieu der Torfmoore, mechanisches und chemisches Verhalten des Nebengesteins der Flöze wirken oft bestimmend auf den Reifegrad (Inkohlungsgrad) einer Kohle. Für jeden dieser Haupt- und Nebenfaktoren gibt es überzeugende Belege; umstritten ist immer wieder, welchem von ihnen die wichtigste Rolle zuzuschreiben ist, da meist mehrere zusammenwirken. Es scheint, dass in manchen Gebieten der eine, in anderen ein anderer Faktor den grössten Einfluss ausgeübt hat. Die folgenden Betrachtungen seien auf die Wirkung des gebirgsbildenden Druckes auf die Kohle beschränkt.

Nicht anzuzweifeln ist die Wirkung der tektonischen Kräfte auf das Gefüge der Kohle im grossen und im kleinen. Tektonische Verdickungen und Verdrückungen von Kohlenflözen sind aus vielen Gebieten bekannt; sie zeigen, dass sich die Kohle gegenüber ihrem Nebengestein zumeist als ein mobilverformbares, «plastisches»

Gestein verhält. Besonders instruktive Beispiele sind dargestellt worden von FABRE und FLEYS¹, FEUGUEUR² (Abb. 1) und früher von WALTHER E. PETRASCHECK³.

Diese Plastizität ist aber zumeist nur eine scheinbare gewesen, indem die Verformung in Wirklichkeit in einer differentiellen Bewegung kleiner, fester Kohlentelchen bestand, das heisst also in einem Fließen zerriebener Feinkohle (Kohlenmylonit). Die Kohle selbst hat ihren chemischen Reifegrad – etwa ausgedrückt im Gehalt der flüchtigen Bestandteile – schon vor der Durchbewegung und Mylonitbildung besessen. Die Inkohlung war präkinetisch. Das mikroskopische Gefüge ist kataklastisch oder blätterig geschiefert. Gefügebilder dieser Art sind von niederschlesischen Kohlen abgebildet worden (zum Beispiel von HOEHNE⁴ und kürzlich auch von solchen aus dem Ruhrgebiet (M. und R. TEICHMÜLLER⁵, 1954). Feine Kohlenmylonite sind auch aus den Triaskohlen der Alpen und den Kreidekohlen der Balkanhalbinsel bekannt. Verbreitet ist diese Mylonitbildung immer dort,

¹ J. FABRE und R. FLEYS, *Phénomènes de plasticité et migration dans les charbons alpins*, C. r. 19^e Congr. Géol. internat. Alger, 149 (1953).

² L. FEUGUEUR, *Comportement du charbon, dans une nappe helvétique des alpes françaises*, C. r. 19^e Congr. Géol. internat., 3, 163 Alger (1953).

³ WALTHER E. PETRASCHECK, jun., *Verdickungen und Verdrückungen von Kohlenflözen und die Gesetzmässigkeiten ihrer Lage*, Z. prakt. Geol. 45, 172 (1937).

⁴ R. HOEHNE, *Zusammenhang von Mikrogefüge und tektonischer Bewegung bei einer niederschlesischen Kohle* – Glück auf 18, Essen (1934).

⁵ R. und M. TEICHMÜLLER, *Zur mikrotektonischen Verformung der Kohle*, Geol. Jb. Hannover 69, 263 (1954).

¹ Institut für Geologie und Lagerstättenlehre der Montanistischen Hochschule Leoben (Steiermark).