NEW METHODS

A METHOD FOR THE GRAFTING OF A SECOND HEAD ON A FROG

N. P. Sinitsin

From the Department of Pharmacology of the Gorky Medical Institute (Chairman: Prof. N. P. Sinitsin).

(Received September 24, 1955. Presented by Acting Member of The Acad. Med. Sci. USSR V. V. Zakusov)

In the study of the problem of neuro-humoral regulation in the organism, the method of grafting organs and including them in the general stream of the blood circulation in experimental animals to serve as biological indicators is widely employed.

Starting from 1933, our laboratory, in studying various questions of neuro-humoral regulation in the organism, worked out a number of new methods for grafting a second heart on to a frog, rabbit, cat and dog [1, 2, 3, 4]. For these purposes, the method of animating the isolated head of the vertebrates was successfully used and published in the literature: experiments on fish (A. A. Kulyabko, 1902), experiments on dogs [5, 7].

Already in 1937, we applied the method of grafting a second head on to a dog according to Heimans [5] for the purpose of detecting in the venous blood flowing from the head, chemical substances, and of mediators at the moment of stimulating the sense organs of the transplanted head, and discovered in the outflowing blood chemical substances which clearly increased the blood pressure in the dog-donor [6].

Now for these purposes, we have worked out the method described below of grafting a second head on to a frog.

First stage. Under a general ether anaesthesia in the frog (Rana esculenta), the skin over the sternum was cut off in the shape of a circle with 3 cm diameter. Then the whole sternum was removed up to the sternoclavicular joint, after opening the thorax over the heart: the pericardium was cut away, and the frenum of the heart was trimmed. The left arch of the aorta was exposed up to the ramification on the artery, two ligatures were applied in the region of the ramification and the artery cut across. On the central stump of the arch of the aorta, an elastic clamp was placed at the root, and a celloidin cannula was placed on it. The vessel was dissected, and turned back on the cannula in the form of a cuff, in line with our method [1]. Then the upper right vena cava was exposed; two ligatures were applied as far as possible form the venous sinus, and the vein cut across. The operational field was abundantly irrigated with Ringer solution, and covered with a damp gauze napkin.

Second stage. In the second frog (Rana esculenta) of the same size under general anaesthesia, the sternum was widely dissected, the pericardium and frenum were cut away, and all the vessels of the shoulder girdle were carefully ligatured, both left and right, and cut across. Then all the cervical-shoulder muscles up to the vertebral column were cut across left and right ; with a circular cut, the skin around the neck was separated completely from the skin of the torso; the inferior vena cava was carefully exposed, and at the liver itself, it was ligatured and cut across; the left and right lung were freed, they were separately ligatured and removed ; the esophagus was cut across, both descending arches of the aorta were ligatured as high as possible to the head and cut across. The remaining soft tissues connecting the head to the trunk were cut out, and finally, between the second and third vertebrae, the verterbral column was cut across. Hemorrhage from the vessels of the spinal canal was stopped with a tampon of melted wax.

Thus, the head of the frog was separated from the trunk, together with the heart, and the vessels feeding the head ;

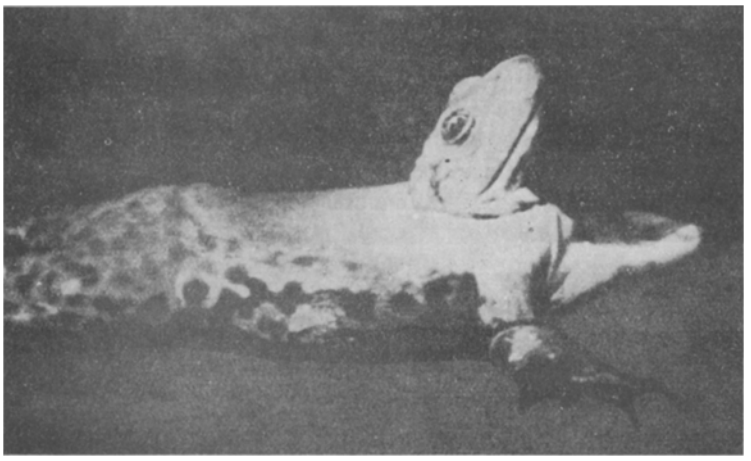

Fig. 1. Grafted frog's head (Rana esculenta) 24 hours after operation.

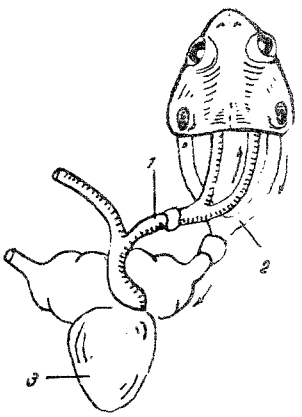

Fig. 2. Diagram of blood circulation in grafted frog's head. 1) aorta, 2) vein, 3) heart.

the arterial cone (bulbus aorticus) was carefully exposed, ligatured at the base, and cut off from heart. On the inferior vena cava, a celloidin cannula was placed and after dissecting out the vessel, it was turned back in the form of a cuff in order to form an anastomsis with the right superior vena cava of the frog-donor ; ligatures were placed on the auricles, which were cut away.

Third stage. The cut-off head of the second frog was transferred to the breast of the frog-donor, and placed on wadded tampons in the forepart (Fig. 1). First of all, an anastomosis was formed between the exposed left arch of the aorta of the donor, and the arterial cone of the transplanted head. For this, the arterial cone was cut open, and placed on the stump of the left arch of the aorta of the frog-donor. Then the anastomosis was formed between the inferior vena cava of the isolated head and the right vena cava of the donor, or between the inferior vena cava and the left auricle by our method [3]. When the anastomosis was correctly formed, the blood swiftly filled both arches of the aorta of the isolated head, and entered the vessels of the brain. Through the anastomosis, between the yenous sinus and the right superior vena cava blood emerged freely from the head into the venous sinus of the heart of the frog-donor. Thus, the blood-carrying system of the grafted head of the frog was included in the major blood circulation of the frog-donor (Fig. 2).

At first, the grafted head was in a state of post-operative shock, but within 5-10 minutes, began to show the first signs of life. The eyes gradually opened, and with a slight touch again closed. 30-60 minutes after the operation, the head, completely recovered from shock, reacted sharply to touch of skin layers, responding with twitching and contraction of the remaining muscles of the neck and head, and with protrusion of the eyes and blinking.

In our experiments, the grafted head of the frog lived more than 48 hours, but we hope that with a perfected method, the survival time of the grafted head can be extended considerably.

The method described above offers wide possiblilites for various observations on a grafted head of the frog. With the aidof a stereoscopic microscope or binocular lens, one can observe blood circulation, and various media of the eyes of the frog. After first cutting out the temporal parietal bones in the grafted head, one can observe the brain blood circulation, and by means of vital dyes, one can study directly the substance of the brain with various intoxications (alcohol, nicotine, etc.), and the effect of various pharmacological substances such as antidotes. The frogs with two heads, undoubtedly, can be used for solving various problems of neurohumoral regulation in the organism. We have described only a few examples from the field of experimental pharmacology, but there are many more in other departments of experimental medicine and biology.

LITERATURE CITED

[1] S. S. Bryukhomenko and S. I. Chechulin, Study of New Methods of Artificial Blood Circulation and Blood Transfusion* (1928) pp. 7-43.

[2] N. P. Sinitsin, Heart Grafting as a New Method in Experimental Biology and Medicine * (Moscow, 1948).

[3] N. P. Sinitsin,Uspekhi Sovremennoi Biol. Vol. 19, No. 2, pp. 277-280 (1945).

[4] N. P. Sinitsin, Byull. Eksptl. Biol. i Med. Vol. 11, No. 3, pp. 255-256 (1941).

[5] N. P. Sinitsin, Klin. Med. Vol. 31, No. 7, pp. 5-14 (1953).

[6] N. P. Sinitsin,Byull. Eksptl. Biol.i Med. Vol. 16, No. 9, pp. 26-28 (1943).

* In Russian.