Regeneration of heart muscle in adult rats
during cobalt-compound action

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REGENERATION OF HEART MUSCLE IN ADULT RATS DURING COBALT-COMPOUND ACTION

By

M. Kh. Klibelei

(Paper presented by Acad. E.M. Kreps, 24/3/75)

In adult mammals during the normal healing process in the area of a myocardial necrosis no regeneration of the muscle fibres of the heart occurs. In the region of the replacing scar tissue at the site of the heart muscle necrosis the regenerating vessels are reduced (3, 10). This may lead to a recurrence of the tissue necrosis. The size of the area affected, the ischemia of the necrotic and scarring area (10), the special characteristics and effect of the dead part of the tissues (11), all determine and limit the efficiency of the heart. For this reason, Academician A.N. Bakulev in his address at the 20th Session of the USSR Academy of Medical Sciences, called the problem of stimulating myocardial regeneration one of the pressing issues in present-day cardiology (2).

*Numbers in the right-hand margin indicate the corresponding pages in the original.
The problem of reparative regeneration of muscles and vessels in the area of a myocardial necrosis attracts many research workers. The surgical methods of revascularization, which have been developed clinically (5), do improve the blood supply to the ischemic area of the heart, but the operations which have been developed cannot be performed on all patients and are only palliatives (4). Spontaneous regeneration of the heart muscle fibres can only be studied in animals which are in the early phylogenetic and ontogenetic stages, mainly frogs and new-born mammals. In adult mammals the regeneration process in the heart muscle fibres (1, 6, 7, 8, 9) and vessels (3) can only be found where the animals have been stimulated with biologically active agents. The authors of these studies point out that further research to find agents capable of stimulating the process of reparative myocardial regeneration in adult mammals is needed. This would enable us to bring about and to study extensively the process of stimulated myocardial regeneration in adult mammals. We could then recommend the most effective agent for the clinical treatment of myocardial infarct, this being the main task where the question of myocardial regeneration is concerned.

In previous experimental work the process of stimulated regeneration of the heart muscle fibres was brought about by treating the animals with a myocardial hydrolysate, with pyrogenal, RNK, dybazol, etc., and also with Vitamin B\textsubscript{12}, which contains the trace element cobalt as its main component. In our research, we assumed that other complex compounds which included cobalt would also be capable of inducing the heart muscle fibre regeneration process. Such cobalt compounds had been used experimentally and clinically to stimulate hematopoiesis. We decided to put them to a new use.

In 180 non-linear white rats, weighing between 300 and 400 grams, using the standard method, (6), we brought about electrodidiathermal coagulation in the cone-shaped part of the wall of the left ventricle of the heart,
in which the muscle fibres and the walls of the vessels were fused and the lumen of the vessels was blocked with a plug of coagulated blood. In the cone of the tissue necrosis, 5 mm in diameter at the base and with the tip of the cone extending to the endocardium, no live muscle nor vessels capable of passing blood remained. There was neither orthograde nor retrograde circulation of the blood. Electrocardiograms were made of the rats while they were under chloral hydrate narcosis. On the ECG, after coagulation of the myocardium, statistically significant changes occurred in the basic indices. The heart beat slowed down, the systolic index was up, the height of the R waves was down in both the standard and chest leads. The lengthening of the PQ interval was statistically insignificant and on the ECG curve the Q wave, which was previously absent, appeared. The curve took on a monophasic appearance.

Beginning immediately after the operation, daily in the course of the first month and every other day throughout the whole of the second month, three (test) groups of rats were given intramuscular injections of cobalt compounds, while a control group was given a physiological salt solution. The first test group of rats was given a 1% solution of "coamide", the second group a 0.4% solution of "Co-35", while the third group was given a 1% solution of "Mixture-1". The preparations were given in doses of 3 to 5 mg per kg of rat body weight. On days 3, 7, 13, 20, 35, 60 and 140 of the experiment some rats were decapitated, the hearts were fixed in 12% neutral formalin. It was possible to detect the presence or absence of elements of muscle in the area of the myocardial scarring without resorting to the isotopic autoradiography or the electronic microscope, needed in tracing the source of the myoblasts which are developing into heart muscle fibres. As regards the novel and controversial aspect of the source of myoblasts, it was our opinion that in research work this question could not
be the subject of a parallel investigation, but rather, needed independent examination. Therefore, the special methods of investigation mentioned above were not used. In order to detect muscle elements in the area of scarring, we used the adequate and widely used method of staining histological sections. Celloidin sections, 10 - 12 microns thick, were stained with Weigert's hematoxylin, ferric hematoxylin, and counter-stained with eosin and picrofuchsin using van Gieson's method. Consequently, at each stage of the experiment, the main ECG indices of the rats were studied and the quantitative data were statistically processed. Thus we were able to judge both the morphological changes as well as the electrophysiological condition of the heart in rats of both the control and the test groups.

In the control series of the experiment, the development of granulations, lysis and the resorption of the necrotized tissue mass were all slow, and the damaged area was replaced only with scar tissue. The muscle stumps in the damaged area retained their ragged appearance. There was no enlargement, no duplication of the nuclei, no change in striation and no signs of dedifferentiation. Instead, the terminal segments of the muscle stumps gradually disintegrated and the area of scarring expanded. In the control group, the revascularization of the area which was in the process of scarring was paradoxical in character. Vessels did appear, but as early as one month later the arteries began to look as if they were closing. The branches of such arteries did close as a result of pathological changes in the intima. Sixty days after the operation necrosis of the tissues began to recur and after 140 days aneurysmal thinning of the scarred area occurred. The data from the ECG analysis matched the data from the morphological studies. So, up to the end of the experiment in the control group, the scarring form of the ECG was maintained, the Q wave did not disappear, the statistically
significant drop in the height of the R wave was maintained in the chest lead. There was a rise in the systolic index and an increase in the duration of the ventricular complex which had arisen as a result of the coagulation of the myocardium. The variation in the PQ interval proved to be statistically insignificant in comparison to the original interval, as did the height of the R waves in the standard leads. Thus, there was no restoration of the original indices or of the ECG forms in the control group.

The best results were achieved in the experiment with the "Co-35" compound. In this experiment, even after three days the main mass of dead tissues was encircled on all sides and permeated by granulations. After 13 days there were no longer any undisintegrated dead muscle fibres. In the stumps of the muscle fibres, cytoplasm was beginning to show through, the nuclei were growing larger, were dividing and contained several large nucleoli. At the site of the segment of the muscle fibre, there appeared, along with the stumps, spindle-like cells with large nuclei which were lying at random, in groups and chains. Among the scattered, undifferentiated cells mitotic figures were to be seen. Mitotic division of undifferentiated cells occurred after three days, was active after seven days and after 13 days was found among the random cells in the areas where the granulations later developed. After cell aggregation the mitoses stopped. If, after three days, random, spindle-like, undifferentiated cells apparently moving out of the stumps of dedifferentiating muscle fibres could be seen, cells which in seven days (fig. 1 a, see inset to page 227)* built up in the form of groups and chains, then in 13 days (fig. 1 b)* in the region where healing was taking place, one could see concurrently both random, spindle-like cells and groups of cells, mitotically dividing, and symplastic formations and tubules. After 20 days, fine muscle fibrils appeared in the developing

*Translator's note: no copy of the illustrations, which are in fact in another part of the journal (page 227), was included with photocopy of the text.
connective tissue. These fibrils contained several nuclei and, when stained with picrofuchsin using van Gieden's method, they took on a specifically yellow colour. After 35 days (fig. 1 c) along with the newly formed arteries, syncytially fused, only slightly differentiated muscle fibres had appeared in the connective tissue. These fibres stained a specifically yellow colour and had nuclei, delicate striation and intercalary laminae which run obliquely. After 60 days, along with the fully-fledged artery, a syncytial layer of differentiated muscle fibres lay in the connective tissue. It had normal nuclei, and longitudinal and transverse striation. The syncytial layer of regenerated muscle tissue survived along with the fully-fledged, regenerated artery in the connective tissue even after the 140th day of the test (fig. 1 d). Staining with ferric hematoxylin brought out the fibrillation and striation of the muscle fibre cytoplasm of the regenerative agent very well. The particular characteristics of the course of the morphological transformations in the area of the damage to the heart wall in rats treated with "Co-35" may be linked with the features which are characteristic of the revascularization of this area. Following the rapid takeover of the damaged area by granulations, the newly forming vessels quickly differentiated and the necessary layers of vessel walls appeared in them. There was no gradual expansion of the intimal cell layer or closing of the vessel lumen, neither was there a recurrence of the tissue necrosis nor the formation of aneurisms.

The data of the morphological study concurred with that of the ECG. As early as three days after coagulation and until the end of the experiment there was no difference between the initial data and the later as regards heart-beat rhythm and the duration of atrioventricular conduction. After only 7 days, the height of the R wave in the standard lead was statistically
insignificant in its differences from the original height. Normalization of the duration of the ventricular complex began after 13 days, while normalization of the systolic index started 20 days after the operation. However, the approximation of the height of the R wave in the chest lead only began after 35 days and the position of the ST segment remained inverse or biphasic until the 35th day. After only three days the shape of the ECG curve changed from monophasic to biphasic. The Q wave also disappeared - this may be linked with, (a), the fact that on all sides granulations rapidly took over the mass of coagulated tissues, (b), with the particular characteristics of the revascularization of the damaged area, and (c), with the fact that along with the connective tissue, gradually developing, and slowly differentiating heart muscle fibres, lying alongside the fully-fledged, regenerated arteries, also appeared. The rapid normalization of the ECG in animals where the regeneration of the myocardium has been stimulated has been reported in other studies (1, 6).

When they retracted, the mature muscles of the outer areas could not stimulate all the transitional forms of slowly differentiating muscle tissue which relieve each other chronologically. At no stage, neither in the control group nor in the test group, was any muscle tissue which had survived the electrosurgicalization found (the conditions under which the damage was inflicted exclude this possibility). Therefore we had no reason to believe that, in the process of induced regeneration of the myocardium which we were observing, we were dealing with muscles which had either survived or been drawn into the damaged area. We found nothing similar in the control group. Our data on the process of stimulated regeneration of the myocardium as we observed it, agree with the limited data in previously published studies where other agents were employed to stimulate the regeneration process in the myocardium of mature mammals. As a result of
our research, we suggest that, along with other agents which have been
used to stimulate myocardial regeneration, the preparation "Co-35",
which has previously been employed both experimentally and clinically to
stimulate hematopoiesis, may also be used.

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BIBLIOGRAPHY

1. S.V. Andreev, A.V. Dokukina and others. Experimental Therapy
in Cardio-vascular Diseases, Moscow, "Meditsina", 1968.

2. A.N. Bakulev. The Most Important Problems. Meditsinskaya gazeta,


4. Kh. N. Muratova. Surgical Treatment in Cases of Chronic Coronary


6. L.V. Polezhaev. The Loss and Recovery of Organ and Tissue

7. S.S. Saidrasulov. Izvestie of the USSR Academy of Sciences,

8. N.P. Sinitsyn. In the book, "Regeneration and Cell Division",

9. N.D. Skuba. Tr. of the 1st All-Union Congress of Anatomico-

10. A.V. Smol'yaninkov, T.A. Naddachma. The Pathological Anatomy
of Coronary Insufficiency, Moscow, 1963.

11. M.G. Udelen'nov, E.Ya. Kyandzhuntseva and others. Tr. of the 14th
All-Union Congress of Therapists, Moscow, "Medgiz", 1958.

ЦИТИРОВАННАЯ ЛИТЕРАТУРА

1 С. В. Андреев, А. В. Докукина и др. Экспериментальная терапия сердечно-
сосудистых заболеваний, М., "Медицина", 1968. 2 А. Н. Бакуле. Вышебийные пробле-
мы. Медицинская газета, № 2320, от 3 июля 1964 г. 3 М. Х. Клибле. Кровооб-
5 М. Плот, Коронарная болезнь, М., 1963. 6 Л. Б. Полежаева. Утрата и восстановле-
ние регенерационной способности органов и тканей у животных, М., "Наука", 1968.
В кн. Регенерация и клеточное деление, М., 1968, стр. 384. 9 Н. Д. Скуб, Ц. 1 съез-
да патологоанатомов УкнССР, Киев, "Здоровье", 1971, стр. 257. 10 Л. В. Смольянин-
ников, Т. А. Наджинкина. Патологическая анатомия коронарных нарушений, М.,
1963. 11 М. Г. Уделен'нов, Е. Я. Канжунцева и др., Ц. XIV Весенних съездов тера-
певтов, М., "Медицина", 1958.