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Dalsze badania nad wpływem pobierania krwi od karpi na ich obraz krwi i przyrosty — Further investigations on the influence of blood sampling in Carp on their blood picture and rate of growth

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Investigations of the blood of fish are still of an experimental character. One of the main difficulties of these investigations is the taking of an adequate amount of blood from living individuals often not more than 8—10 cm long. Besides, it is known that fish have a very small amount of blood; the total blood volume amounts on an average 1/50 of weight of body. The opinion is maintained, that the collecting of an adequate amount of blood for conducting basic haematological determinations shall cause, after a certain time, the death of the fish or at least inhibit its rate of growth.

The method of taking blood samples by means of glass canules proposed by Pučkov (1954) allows to take an adequate blood sample from small specimens without causing permanent damage of the investigated subject and thus enabling a repetition of sampling after a short lapse of time.

Haematological researches of fish are laborious and need technical equipment not always attainable in the country (high-speed centrifuge, colorimeters, a set of melangers and counting chambers etc.). Molnár and coll. (1956) proposed, with the aim of simplifying work during investigation, that haematocrit values be given only in the final results instead of the number of erythrocytes in 1 mm^3 and haemoglobin percentage content. This method however does not solve the technical difficulties and is not precise enough. Philips, Van Slyke and coll. (1950) state that when the radius on which blood samples were centrifugated is dimindshed by half, it provokes a change of about 8% in haematocrit value (0,51 by r-9 cm and 0,47 by r-18 cm). Gregersen, Schiro (1938) after these authors ascertained that a column of blood morphotic elements centrifugated by the standard method, still has up to 7% of plasma remaining on its surface because of adhesion. Snieszko and coll. (1960) carried out investigations on trout and proposed introducing the microhaematocrit (that requires only a very small amount of blood), as a haematological test, still with the reservation that these values may serve as normal for trout until more extensive data are collected. The author reviews also literature concerning this problem.

This work is the continuation of investigations undertaken in 1957 (Łysak 1959 a) with the aim of ascertaining the influence of collecting small quantities of blood from carp upon their rate of growth and, later, on their blood picture. At present, results obtained during the course of investigations in the next two years shall provide a larger and more positive base for drawing conclusions.

It was necessary to find a simple, universal and rapid method which would allow to accomplish in field conditions and in the same space of time, a greater amount of estimations as accurately as possible. The possibility of a more exact assessment of these values lies mostly with the increasing of the number of investigated samples, as some haematological factors are greatly influenced by their surroundings and their dispersion is considerable in individuals living in identical conditions of environment. I therefore turned towards the gravimetric method of estimation of the density of blood and plasma by means of standard solutions of copper sulphate (Philips, Van Slyke, Hamilton, Dole, Emerson, Archibald 1950 a,c). A description of this method for investigation of fish blood can be also found in Privolniev (1959).

Material and method

Investigations were carried out in the years 1958—1959 in the Fishery Experimental Station at Mydlniki, belonging to the Agricultural College in Kraków. Mirror carp in their second and third year of life $K_{1/2}$ and $K_{2/3}$) were examined in four experimental ponds (table I).

Tab. I

List of ponds and fish material

Year	Pond		Fish		Feeding
	name	surface m^2	age	number	
1958	Konrad	1000	$K_{1/2}$	110	natural food
	Spytek	1000	$K_{2/3}$	110	
1959	Mieszko	1000	$K_{2/3}$	110	barley
	Migiel	500	$K_{2/3}$	75	

Moreover, in the autumn of 1959 in 10 ponds of the group „Za Młynówką" I took (only once) blood samples from 250 specimens of carp in their second and third year of age. In order to increase the number of observations when working out the diagram for estimation of blood density, data obtained in the period of time between April and October 1959 from fish stocking of the same group of ponds have been used. Every year in spring (beginning of May) the whole stocking material for experimental ponds was measured (body length, length of opercle, the greatest and the smallest height, and the greatest thickness of the body and the individual body weight were noted). Blood was sampled from one half of the individuals in the quantity of about 0,1—0,3 ml from each specimen. Following biometrical measurements of whole stock and blood samples from 20 specimens out of each pond ensued after 14, 28 and 42 days from the moment the first blood sample had been taken, in mid-summer (end of July - beginning of August), and in autumn before the final fishing (first half of October). Each time I took blood samples from 10 fish that had been sampled during stocking (the so-called experimental group) and for comparison, blood was taken from another 10 fish that had not been sampled (the so-called control group). The scheme of blood sampling was the same for every one pond (table II).

Tab. II.

Schematic plan of fishing and blood taking

Experimental group							Control group						
Number of fish	Blood taking No						Number of fish	Blood taking No					
	I	II	III	IV	V	VI		I	II	III	IV	V	VI
1-10	+	+					11-20	-	+				
21-30	+		+				31-40	-		+			
41-50	+			+			51-60	-			+		
61-70	+				+		71-80	-				+	
81-90	+					+	91-100	-					+
Additional control group													
	-						101-110	-					

In the ponds Konrad, Spytek, Mieszko, Migdał the fish were marked with silver tags attached to the base of the dorsal fin, behind its first ray. In 1958 dyed nylon thread was used for attaching the tags (dyeing had been aplied for easier identification of groups-every group had a different colour of thread). Nylon, in spite of special knots that had been applied, had a tendency of becoming untied and the apertures through which it passed healed with difficulty. In 1959 nylon was replaced by silver wire, which was more suitable in this respect.

All fishings were conducted at the same time of the day (morning hours). I also carried through simultaneous measurements of temperature and water oxidation, marked on the tables containing results of haematological estimations.

I estimated in collected blood samples: the number of erythrocytes per 1 mm^3 in T h o m a - Z e i s s chambers; percentage of haemoglobin contents in Sahli's haematometre (100% = 16 g of haemoglobin in 100 ml of blood); number of white blood corpuscles and also erythrocyte dimensions — microscopically, on smears dyed with the May-Grünwald-Giemsa method; the relation of the volume of morphotic elements to the volume of plasma in haematocrite tubes (centrifugated with a speed of 3500 turns per minute, during half an hour, r-12 cm). Previously, a more detailed description concerning the method was given (Łysak 1959 a, 1959 b, and Łysak, Wójcik 1960). I investigated the specific gravity of the blood by means of the gravimetrical method (Philips, Van Slyke and coll. 1950) consisting in establishing the density of the investigated sample in relation to very precise (prepared with an accuracy to the fourth decimal place) copper sulphate solutions ($CuSO_4 \cdot 5\,H_2O$). For this purpose solutions of other salts denaturing proteins may be used: zinc sulphate or sodium chloride with an addition of picric acid. Solutions of benzen, glycerol or chloroform have been used previously with unsatisfactory results, however, because of considerable divergencies in warmth dilatation coeffecients.

I prepared standard solutions of copper sulphate with the aid of a pycnometre for carp of an age period suitable for the present investigations in the region of density 1,020 to 1,042, at intervals of 0,001 or 0,002 departing from a solution of $CuSO_4$ with a specific weight of 1,100. Privolniev (1959) proposes that for all fish solutions be prepared with a specific weight of the order of 1,032 to 1,051.

I placed portions of 50—100 ml of standard solutions in glass cylinders with a fluid column of 10 cm high (Fig. 1). Then with the aid of a pipette, the blood was dropped succesively into the cylinders. When a certain degree of practice is attained, 3—4 drops of the investigated

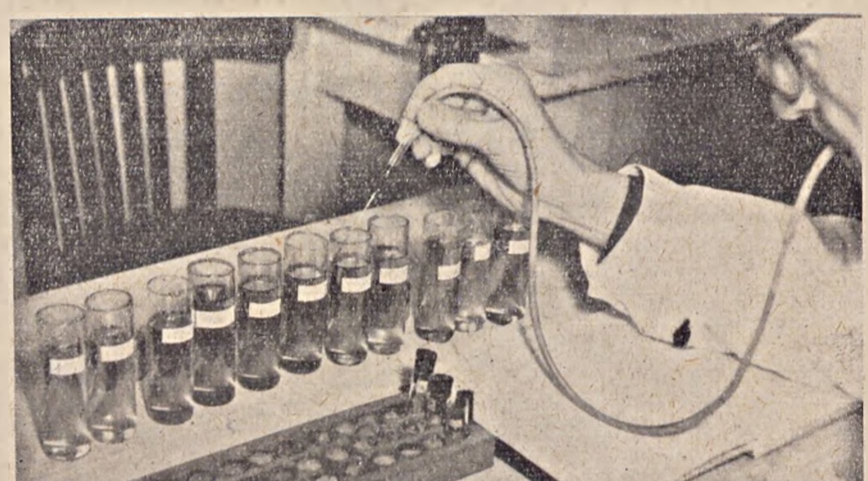

Fig. 1. The setup for gravimetrical estimation of specific gravity of blood.

sample allow to determine its specific gravity. Drops of blood in the copper sulphate solution take the form of truncated cones or small rings and according to the difference arising between their density and that of the standard solution into which they were dropped, they fall to the bottom (lower concentration of $CuSO_4$) or float on the surface of the solution (higher concentration of $CuSO_4$). The cylinder in which a drop of investigated blood will remain for about 10 seconds in the middle part of the column of fluid indicates the density of the sample under investigation. This method demonstrated a great tolerance to thermal conditions, as it is possible, with its aid, to perform estimations in the environmental temperature of 4 to 40 °C without the risk of erroneous results. However the difference between the temperature of the investigated sample and that of the standard solutions cannot be greater than 5 °C.

Standard solutions, within certain limits are automatically clarified, as in the space of 1—2 minutes the introduced material settles on the bottom of the cylinder as a precipitate. A 100 ml portion of standard solutions having received 50 drops of blood (after the performing of 50 estimations) diminishes its specific gravity by 0,0002. A renovation of the solution can be obtained by introducing, after performing 50 estimations, 0,2 ml of the primary copper sulphate solution with a specific gravity of 1,100 (Philips, Van Slyke and coll. 1950).

Results of biometrical measurements

In every pond, after blood had been collected for the third time, that is 28 days after the time of stocking, three groups could be found: the first, being carp from which blood had not been collected yet, the second, of carp from which blood had been collected once; the third, of carp from which blood had been collected twice. The number of fish in these groups varied after each fishing. Carp having blood taken for the first time passed from the first to the second group, those from the second to the third group, while remaining under observation for changes of biometrical features. Specimen losses were as follows:

Pond	Konrad	—	14,3%	($K_{1/2}$)
,,	Spytek	—	0 %	
,,	Mieszko	—	3 %	($K_{2/3}$)
,,	Migdał	—	0 %	

These losses are insignificant, considering that for commercial carp production the following loss is considered as normal: 10—15% for $K_{1/2}$, 5 — 10% for $K_{2/3}$. It must also be stated that there were losses both in the groups from which blood had been collected and in the control ones. Therefore, one ought not to consider that the increase of the loss of specimens was provoked by damaging during the collecting of blood samples, with one restriction however, that the technique of blood taking be conducted faultlessly.

Even when blood from carp in their second and third year of life is collected twice, in amounts of 0,1 to 0,3 ml from each individual, it does not provoke a checking of their growth (tab. III, Fig. 2). More than that, I have already described an insignificant tendency (Łysak 1959 a) of an increase in the rate of growth obserwed in individuals from which blood had been collected in comparison with control specimens (without blood taking). These results are clearly presented in table IV, in which the final, individual weight of fish of the second and third group is submitted in percentage values, suitable weights of the first (control) group being considered as 100. In all cases the figures surpass 100, with the exeption of the final fishing in the pond Mieszko, where the mean weight for the first group surpasses suitable values of the second and third groups. However, the differences between mean individual weights both in plus and minus, worked out by means of the variation analysis method are not statistically significant.

Table V presents the coefficients of the significance of F_0 differences (after the Snedecor-Student tables, for a suitable number of degrees of freedom), and obtained from $F_{emp.}$ calculations for means of final individual weigths. Coefficients for the remaining biometrical measurements are still lower, that is why they are not presented here. These diferences cannot be considered as statistically significant in view of the considerable dispersion of investigated material. However the obtained results permit us to conclude, that even when blood is collected twice from carp ($K_{1/2}$ and $K_{2/3}$) in the amounts given above it does not cause a check in their growth rate. It is quite comprehensible that transgression of certain limits in the amount of collected blood will cause derangement in the physiological balance of the system, which in effect will lead to a diminution of the general resistance of the organism, a weakening of the rate of growth and even to the death of the investigated subject. Larger quantitative samples (about 0,6—1,0 ml) collected from carps in their second or third year of life already provoke a certain checking of growth rate and a decrease in the value of haematological indexes. This can be illustrated by material borrowed from another experiment, also carried through by me in the Fishery Experimental Station at Mydlniki (Agricultural College — Kraków), and presented in Tab. VI. In $K_{1/2}$ the decrease in weight and length of body under the influence of the collecting of blood samples, as mentioned above, amounts to an average of 2,5%, and haematological data (with exeption of haemoglobin percentage content, is subject to very small fluctuations) decrease on the average by 2,4%. In $K_{2/3}$ we observed a small influence on the body length

Tab. III.

Changes in biometrical features under the influence of blood collecting

Group I - no blood collecting, II - onefold blood collecting, III - twofold blood collecting

Year	Age of fish and name of pond	Date	Weight of body I fish quant.	I g	II fish quant.	II g	III fish quant.	III g	Length of body cm I	II	III	Length of opercle cm I	II	III	Height of body maximal cm I	II	III	Height of body minimal cm I	II	III	Thickness of body maximal cm I	II	III
1958	$K_{1/2}$ Konrad	9.V.	60	48	50	49	-	-	11,3	11,4	-	3,5	3,6	-	4,3	4,4	-	1,6	1,6	-	2,1	2,1	-
		23.V.	60	56	49	58	-	-	11,6	11,8	-	3,7	3,8	-	4,6	4,7	-	1,7	1,7	-	2,4	2,4	-
		6.VI.	48	82	48	89	8	99	13,3	13,6	13,9	4,2	4,3	4,4	5,4	5,6	5,8	1,9	1,9	2,0	2,7	2,7	2,8
		20.VI.	33	117	44	107	17	121	14,7	14,4	14,8	4,6	4,5	4,7	6,1	5,8	6,1	2,1	2,1	2,2	3,0	2,9	3,1
		28.VII.	25	289	43	296	24	325	19,8	20,0	20,5	5,9	6,0	6,1	8,3	8,3	8,6	2,9	2,9	3,0	4,0	4,1	4.3
		7.XI.	14	522	42	568	33	582	24,0	24,5	24,7	7,1	7,2	7,3	10,1	10,3	10,6	3,5	3,7	3,7	5,0	5,0	5,1
	$K_{2/3}$ Spytek	3.V.	60	308	50	308	-	-	21,0	21,1	-	6,4	6,5	-	8,2	8,3	-	3,0	3,0	-	3,9	3,9	-
		17.V.	60	321	50	329	-	-	21,2	21,5	-	6,4	6,5	-	8,4	8,5	-	3,0	3,0	-	4,1	4,1	-
		30.V.	42	334	44	356	8	361	21,9	22,2	22,3	6,6	6,6	6,9	8,5	8,6	8,5	3,0	3,1	3,1	4,2	4,2	4,2
		13.VI.	34	368	36	384	15	400	22,6	22,8	23,2	6,8	6,8	6,9	8,8	8,9	9,1	3,1	3,1	3,2	4,2	4,2	4,3
		26.VII.	30	624	38	616	20	630	26,4	26,3	26,6	7,6	7,6	7,7	10,7	10,6	10,6	3,7	3,6	3,6	5,4	5,4	5,4
		4.XI.	20	1017	39	1018	30	1049	30,5	30,6	30,6	8,8	8,7	8,8	12,3	12,3	12,3	4,4	4,5	4,4	6,3	6,3	6,3
1959	$K_{2/3}$ Mieszko	21.IV.	40	274	60	274	-	-	20,4	20,5	-	6,1	6,1	-	7,9	7,9	-	3,0	3,0	-	3,9	3,9	-
		4.V.	60	281	40	285	-	-	20,6	20,5	-	6,1	6,1	-	8,1	8,1	-	3,0	3,0	-	4,0	4,0	-
		18.V.	50	280	40	270	10	298	21,0	21,1	21,0	6,1	6,0	6,2	8,0	7,9	8,2	3,0	2,9	3,1	4,0	3,9	4,1
		2.VI.	38	287	39	290	20	298	21,0	21,1	21,0	6,1	6,2	6,2	7,9	7,9	8,0	3,0	3,0	3,0	4,0	4,0	4,0
		6.VIII	28	709	39	714	25	737	27,1	27,1	27,4	7,6	7,6	7,7	10,8	11,0	11,1	4,0	4,0	4,0	5,6	5,6	5,7
		6.XI.	20	943	39	929	38	931	29,5	29,3	29,2	8,3	8,2	8,2	11,9	11,8	11,8	4,3	4,3	4,3	6,0	5,9	5,9
	$K_{2/3}$ Migiel	25.V.	45	372	30	372	-	-	23,2	23,2	-	6,9	6,9	-	8,7	8,7	-	3,2	3,2	-	4,3	4,3	-
		8.VI.	45	397	30	398	-	-	23,2	23,3	-	6,8	6,8	-	9,0	8,9	-	3,3	3,2	-	4,5	4,5	-
		22.VI.	35	410	30	411	10	408	23,5	23,8	23,7	6,9	6,9	6,8	8,8	8,9	8,8	3,3	3,3	3,2	4,4	4,4	4,4
		8.VIII.	25	605	30	628	20	629	26,1	26,5	26,5	7.5	7,6	7,5	10,1	10,3	10,2	3,8	3,8	3,8	5,4	5,3	5,3
		10.XI.	15	832	30	844	30	856	28,2	28.1	28,4	8,0	7,9	8,0	11,5	11,5	11,6	4,0	4,0	4,0	5,8	5,8	5,8

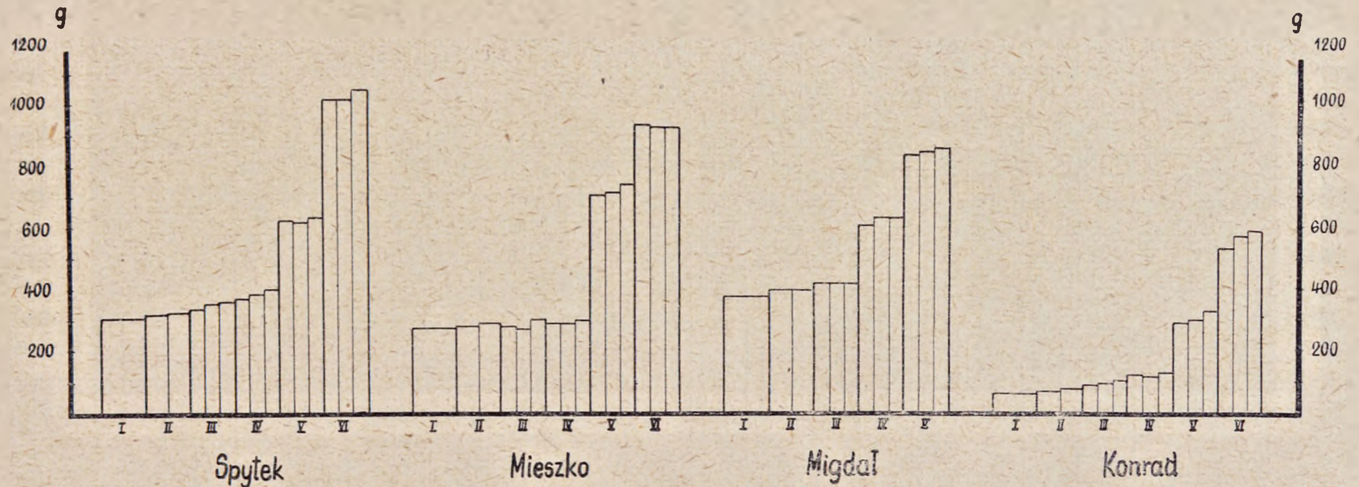

Fig. 2. Changes in the weight of the body of carp (K_{12} and K_{23}) under the influence of blood collecting.

Tab. IV.

The final weight values of carp from which blood samples were collected in comparison with control fish (in %)

Name of pond	No. of blood collect.	Group of fish		
		I	II	III
Konrad	I	100	102,0	-
	II	100	103,5	-
	III	100	108,5	120,7
	IV	100	97,4	103,4
	V	100	102,4	112,4
	VI	100	108,8	111,5
	Mean	100	102,8	112,0
Spytek	I	100	100,0	-
	II	100	102,5	-
	III	100	106,6	108,1
	IV	100	104,3	108,7
	V	100	98,7	101,0
	VI	100	101,1	103,1
	Mean	100	102,0	105,2
Mieszko	I	100	100,0	-
	II	100	101,4	-
	III	100	96,4	106,4
	IV	100	101,0	103,8
	VI	100	100,7	103,9
	VI	100	98,5	98,7
	Mean	100	99,7	103,2
Migdał	I	100	100,0	-
	II	100	100,3	-
	III	100	100,2	99,5
	IV	100	103,8	104,0
	V	100	101,4	102,8
	Mean	100	101,4	102,1

Tab. V.

The comparison of F-coefficients evaluated from the experimental material with suitable coefficients from Snedecor-Student s tables

Name of pond	F emp.	F (from tables)
Konrad	1,290	3,11
Mieszko	0,633	3,11
Spytek	0,382	3,11
Migdał	0,184	3,13

Tab. VI.

Checking of the rate of growth and changes in the blood picture of carp ($K_{1/2}$ and $K_{2/3}$) caused by taking 0,6 - 1,0 ml of blood

\- values for fish, from which the blood wasn't collected

\+ values for fish, from which the blood was collected in quantity 0,6 - 1,0 ml.

Investigated feature	$K_{1/2}$			$K_{2/3}$		
	-	+	reduction %	-	+	reduction %
Body weight	646	626	2,6	1037	997	3,9
Body length	26,86	26,23	2,4	29,58	29,44	0,5
Blood sp.grav.	1,0358	1,0349	2,8	1,0388	1,0377	2,8
Erythr. number	1,360	1,330	2,2	1,425	1,407	1,3
Haematocrit	0,325	0,317	2,5	0,354	0,342	2,8
Percentage of Hb	44,9	44,7	0,5	52,7	50,6	4,1

caused by the collecting of blood samples, there was barely a 0,5% decrease, while the fall in the individual weight is already 3,9%. The cited haematological indexes are lower by 2,9% on the average (but in percentage of haemoglobin content this value is the highest).

Results of haematological research

Numerical data related to haematological indexes are presented collectively on table VII. Here in relation to the smaller number of data (during every fishing, biometrical measurements were conducted on the entire stock, and collecting of blood samples only on 20 individuals from each pond), and also in regard to a greater number of investigated factors, the situation is much more complicated. Worse health conditions of stocking material in the pond Mieszko also tend to render the picture less distinct, and have a certain influence on its later growth increase and picture of the blood composition. In spite of that some dependencies appear quite distinctly. Specific gravity of the blood, number of erythrocytes in 1 mm^3, haemoglobin percentage content and the relation of morphotic elements to the plasma (haematocrit) were as a rule higher in experimental fish (that is those from which blood had been taken in the first fishing), in the period of 14—28 days after the first collecting of blood samples, in comparison with controls. This could prove that this period is sufficient for the fish organism to complete the losses caused by the collecting of blood. The collecting of blood samples also causes a visible increase of white blood corpuscles numbers in a volume unit (sometimes by 60%, as was the case in the pond Mieszko) during the time of 14—28 days in comparison with the corresponding values for control fish. This phenomen can easily be explained by the defensive reaction of the organism against the operation of blood taking. This value in a later period keeps on a level conforming with that of the controls. One can state in general that the number of white blood corpuscles in a unit of volume attains its highest value, for carp in their second and third year of life, in spring and in autumn, with a maximal diminution in the summer months.

In the material I investigated, the collecting of blood samples caused, in the first 14 days (and in the case of fish from the pond Mieszko even after 28 days) an increase of the share of young, spherical forms of erythrocytes in the morphological picture of the blood. The dimensions of red blood corpuscles of the experimental group (second collecting of blood) were as follows: $12{,}54 \times 8{,}75\,\mu$ for $K_{1/2}$ and $11{,}53 \times 8{,}88\,\mu$ for $K_{2/3}$. In the control group (blood was collected for the first time) this value amounted to $12{,}84 \times 8{,}59\,\mu$ for $K_{1/2}$ and $11{,}73 \times 8{,}72\,\mu$ for $K_{2/3}$. In the experimental group of both years erythrocytes had a more spherical form, which would indicate an intensified erythropoiesis process in the specimens. After the already mentioned period of 2—4 weeks the erythrocyte dimensions of the investigated and control groups did not present any distinct divergencies. The shape of erythrocytes, as well as other haematological indexes in fish is greatly influenced by

Tab. VII.

Changes in the blood picture in carp ($K_{1/2}$ and $K_{2/3}$) under the influence of previous blood collecting

– group of fish, from which blood was not collected, + group of fish, from which blood was collected

Age of fish, name of pond	Date	water temp °C	oxygen cont. mg/l	blood sp.grav. –	blood sp.grav. +	erythrocytes mill./mm³ –	erythrocytes mill./mm³ +	dimensions in μ –	dimensions in μ +	surface in μ² –	surface in μ² +	haematocrit –	haematocrit +	% Hb –	% Hb +	white blood corpuscles thous./mm³ –	thous./mm³ +	rel. to erythr. –	rel. to erythr. +
$K_{1/2}$ Konrad	9.V.	10,6	10,0	–	–	–	1,509	–	–	–	–	–	–	–	54,0	–	–	–	–
	23.V.	11,1	12,2	1,0298	1,0299	1,150	1,156	12,84 x 8,59	12,54 x 8,73	172,82	170,73	–	–	35,7	38,8	32,0	45,5	1:35,9	1:25,4
	6.VI.	12,2	11,5	1,0299	1,0300	1,126	1,153	12,30 x 8,74	11,67 x 8,49	158,00	158,80	–	–	32,5	32,5	35,5	54,6	1:31,7	1:25,4
	20.VI.	14,0	10,0	1,0330	1,0309	1,147	1,162	11,62 x 8,79	11,59 x 8,75	160,26	158,44	–	–	34,2	37,0	52,8	69,9	1:21,8	1:16,6
	28.VII	16,5	7,2	1,0361	1,0389	1,525	1,562	11,39 x 8,89	11,31 x 8,86	159,29	157,89	0,343	0,339	48,0	50,2	45,5	45,2	1:33,4	1:34,5
	7.XI.	7,1	8,2	1,0408	1,0408	1,602	1,601	12,01 x 8,26	11,93 x 8,17	156,37	153,20	0,355	0,353	57,0	57,0	63,4	61,3	1:25,2	1:26,1
$K_{2/3}$ Spytko	3.V.	8,1	12,0	–	–	–	1,407	–	–	–	–	–	–	–	43,0	–	–	–	–
	17.V.	12,0	11,2	–	–	1,445	1,479	11,73 x 9,01	11,68 x 8,89	168,99	163,20	–	–	41,1	44,2	85,0	104,0	1:17,0	1:14,2
	30.V.	15,5	9,3	1,0336	1,0334	1,305	1,348	11,86 x 9,38	12,49 x 8,92	175,62	174,66	–	–	41,2	41,6	59,0	58,8	1:22,1	1:22,9
	13.VI.	12,7	7,8	1,0338	1,0356	1,260	1,389	–	–	–	–	–	–	37,9	43,2	–	–	–	–
	26.VII	15,0	6,0	1,0365	1,0376	1,368	1,539	11,82 x 9,17	11,92 x 9,15	170,43	170,01	0,367	0,368	42,6	46,4	73,1	74,2	1:18,7	1:20,7
	4.XI.	7,5	10,2	1,0402	1,0385	1,411	1,407	12,46 x 8,52	12,04 x 8,73	166,81	163,91	0,326	0,357	47,7	47,1	137,6	125,6	1:10,2	1:10,2
$K_{2/3}$ Miasko	21.IV.	8,0	12,8	–	1,0310	–	1,157	–	11,77 x 8,09	–	150,60	–	0,258	–	32,8	–	89,1	–	1:12,9
	4.V.	13,0	13,8	1,0340	1,0300	1,295	1,311	11,98 x 8,29	11,56 x 8,69	156,37	158,44	0,310	0,260	34,9	31,9	89,2	146,2	1:14,5	1: 7,7
	18.V.	–	–	1,0356	1,0349	1,368	1,322	11,66 x 8,62	11,47 x 8,85	157,97	158,88	0,357	0,342	39,9	38,2	64,7	58,5	1:21,1	1:22,6
	2.VI.	15,5	10,4	1,0370	1,0397	1,428	1,594	11,90 x 9,06	12,11 x 8,78	170,01	167,17	0,342	0,374	46,3	50,9	81,2	71,5	1:17,6	1:22,3
	6.VII.	18,0	6,0	1,0392	1,0388	1,492	1,461	12,73 x 9,18	12,40 x 9,23	183,43	179,10	0,371	0,373	49,8	47,4	37,5	43,4	1:39,7	1:33,6
	6.XI.	7,2	10,8	1,0390	1,0390	1,580	1,542	12,15 x 8,53	12,15 x 8,54	162,80	162,80	0,360	0,355	51,8	49,7	62,4	83,6	1:25,3	1:18,4
$K_{2/3}$ Migdał	25.V.	14,5	–	–	1,0333	–	1,292	–	11,43 x 8,34	–	148,55	–	0,322	–	35,9	–	62,9	–	1:20,5
	8.VI.	21,0	12,0	1,0340	1,0310	1,235	1,190	11,49 x 8,88	11,51 x 9,03	160,68	168,14	0,359	0,314	38,0	32,3	62,7	60,6	1:19,7	1:18,1
	22.VI.	16,8	10,8	1,0340	1,0345	1,286	1,333	11,21 x 8,65	11,70 x 8,65	151,22	157,97	0,309	0,317	38,4	38,7	99,0	84,1	1:13,0	1:15,8
	8.VIII.	16,0	8,2	1,0382	1,0382	1,392	1,568	12,16 x 8,71	11,96 x 8,82	165,63	165,79	0,362	0,377	44,1	47,0	51,1	52,3	1:27,2	1:30,0
	10.XI.	8,1	10,4	1,0374	1,0397	1,433	1,469	12,53 x 8,53	12,60 x 8,42	167,80	166,56	0,331	0,367	47,9	51,2	96,2	98,5	1:15,7	1:15,0
$K_{2/3}$ mean	blood collecting 1	–	–	–	1,0321	–	1,285	–	11,60 x 8,21	–	149,57	–	0,290	–	37,3	–	76,0	–	1:16,7
	2	–	–	1,0340	1,0305	1,325	1,327	11,73 x 8,72	11,58 x 8,88	162,01	163,26	0,334	0,277	38,0	36,1	78,9	106,3	1:17,1	1:13,3
	3	–	–	1,0344	1,0343	1,320	1,334	11,58 x 8,98	11,89 x 8,81	161,50	163,83	0,333	0,330	39,8	39,5	74,2	67,1	1:18,7	1:20,4
	4	–	–	1,0354	1,0376	1,344	1,491	–	–	–	–	–	–	42,1	47,0	–	–	–	–
	5	–	–	1,0380	1,0382	1,417	1,523	12,24 x 9,02	12,09 x 9,07	173,49	171,16	0,367	0,379	45,5	46,9	53,9	56,6	1:28,5	1:28,1
	6	–	–	1,0389	1,0391	1,475	1,472	12,38 x 8,53	12,26 x 8,56	165,80	164,42	0,339	0,360	49,1	49,3	98,7	102,6	1:17,1	1:14,5

environmental conditions and is apt to fluctuate under the impact of many factors. N u s e n b a u m (1953) shows the difference in dimensions of the erythrocytes in salmon entering the river from the sea (14,7 × 10,1 μ) and salmon investigated at the source of the river in the pre-spawning period (15,9 × 9,1 μ). Likewise, I could ascertain (Ł y s a k 1959 b) the existence of small differences in erythrocyte dimensions in small whitefish (*Coregonus albula* L.) bred in pond condition in southern Poland (15,64 × 9,74 μ) and in small whitefish living in lakes in the northern parts of Poland (15,51 × 9,56 μ). The surface of single erythrocytes, both in $K_{1/2}$ and $K_{2/3}$ does not show any significant differences as a result of blood collecting.

Values presenting the specific weight of blood show very distinctly a considerable accordance of the changes with fluctuations to which

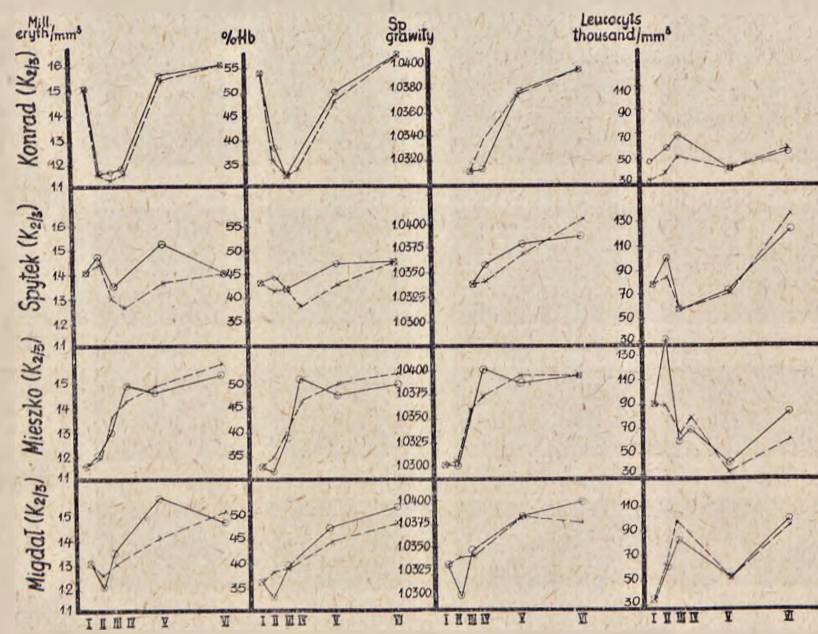

Fig. 3. Changes in the number of red and white blood corpuscles, haemoglobin content, and haematocrit of carp under the influence of previous blood collecting. ——— experiment group, — — — — control group.

the number of erythrocytes in a volume unit, percentage of haemoglobin content and haematocrit are subjected (Fig. 3). This coincidence is shown still more distinctly by the high correlation coefficients r (Tab. VIII) indicating an important accordance of changes in these four factors. This accordance permits to simplify considerably the haematological determinations mentioned above, as it suffices to know

Tab. VIII.

Coefficients of correlation /r/, and regression /b/ among the specific gravity of blood /x/, and number of erythrocytes /y/, haemoglobin content /z/, and haematocrit /w/.

x - blood specific gravity, y - number of erythrocytes /1 mm^3

z - percentage haemoglobin content /Hb/, w - haematocrit /Ht/.

	Year	Name of pond	correlation coefficient			regression coefficient					
			$r_{x:y}$	$r_{x:z}$	$r_{x:w}$	b_{xy}		b_{xz}		b_{xw}	
						mill.erythr. for unit x	tg value	% Hb for unit x	tg value	unit of Ht for unit x	tg value
$K_1/2$	1958	Konrad	0,7245	0,7456	-	0,0468	25°	2,055	11°40'	-	-
	1959	block "Za Młynówką"	0,6712	0,7024	0,7450	0,0395	21°40'	1,977	11°10'	0,0104	6°
	Mean		0,6978	0,7240	0,7450	0,0431	23°20'	2,016	11°30'	-	-
$K_2/3$	1958	Spytek	0,8102	0,8004	-	0,0402	22°	1,852	10°30'	-	-
	1959	Mieszko	0,7321	0,6923	0,7124	0,0449	24°	2,272	12°50'	0,0098	5°40'
	-"-	Migdał	0,7040	0,6637	0,7328	0,0409	22°	2,070	11°40'	0,0101	5°50'
	-"-	block "Za Młynówką"	0,7185	0,8930	0,6630	0,0453	24°	2,280	12°50'	0,0134	7°30'
	Mean		0,7412	0,7624	0,7020	0,0428	23°	2,118	12°	0,0111	6°20'

the value of one of the factors only to be able to read the remaining values, relative to the given blood sample, from the previously elaborated diagram. Disposing of a rather big material from several ponds for a two year period of observation I could work out empirically such a diagram for the carp that I investigated. It appears that dependence of the number of erythrocytes for a unit of volume, of the percentage of haemoglobin content and of haematocrit in relation to specific gravity of blood assumes the shape of a straight-line function $y = a + bx$ in which **a** indicates the distance from zero point of the coordinantes system at which the investigated line intersects the vertical axis and **b** is the tangent of the angle at which it passes in relation to the horizontal axis of the coordinantes system. The **b** value calculated from this equation is at the same time a coefficient of regression, demonstrating how much the dependent variable (y) changes, when the independent variable (x) changes into a unit.

I calculated these coefficients for different groups of carp (in relation to their number in the ponds, kind of food, and to the pond they were stocked in) in their second and third year of life, which I observed in 1958 and 1959. For the independent variable (x) specific gravity of blood was used, and for the dependent variable (y) — successively: the number of erythrocytes in a unit of volume, percentage of haemoglobin content, and haematocrit (Tab. VIII). As regression coefficients for $K_{1/2}$ and $K_{2/3}$ in sundry groups differ only slightly, I combined material for observation of both these years for working out a diagram. I obtained in this manner four rows of augmenting values corresponding to the specific gravity of blood, the number of erythrocytes in 1 mm³, percentage haemoglobin content, and haematocrit (Tab. IX), from the same blood samples of fish in their second and third year of life and from different ponds, in the years 1958—1959. Regression coefficient for these values can be calculated from the formulas:

$$b_{xy} = \frac{S_x^2}{S_{xy}}$$

$$S_x^2 = S\,X - C_x \qquad X = \text{variable independent}$$

$$S_{xy} = S\,XY - C_{xy} \qquad Y = \text{variable dependent}$$

$$C_x = \frac{(SX)}{n}$$

$$C_{xy} = \frac{(SX)\,(SY)}{n}$$

The entire material under observation amounts to: $b_{xy} = 0{,}046$, $b_{xz} = 2{,}009$, $b_{xw} = 1{,}047$. Having now all data and supplementing them with suitable scales I could work out the diagram presented in fig. 4.

It is comprehensible that, as it was elaborated on the basis of observations carried out on fish living in definite environmental

Tab. IX.

Dependence of number of erythrocytes, haemoglobin content and haematocrit on specific gravity of blood in carp ($K_{1/2}$ and $K_{2/3}$)

Blood specific gravity	Number of erythr.		Hb content		Haematocrit		Quantity of fish
	mill/mm³	±	%	±	unit	±	
1,030	1,087	0,132	33,2	3,96	0,284	0,0080	27
1,031	1,131	0,104	34,3	2,60	0,283	0,0090	18
1,032	1,174	0,109	35,6	2,90	0,299	0,0091	43
1,033	1,208	0,108	37,1	2,30	0,308	0,0052	48
1,034	1,281	0,090	39,5	3,21	0,310	0,0088	62
1,035	1,299	0,093	40,7	2,56	0,330	0,0068	77
1,036	1,386	0,102	43,1	4,01	0,337	0,0041	140
1,037	1,418	0,081	45,8	3,82	0,344	0,0056	69
1,038	1,439	0,073	47,2	3,12	0,350	0,0042	73
1,039	1,460	0,081	47,7	3,18	0,363	0,0085	94
1,040	1,521	0,090	51,9	3,48	0,381	0,0093	76
1,041	1,648	0,102	56,8	3,23	0,406	0,0078	68

conditions, in conditions approximately the same (climate, breeding etc.), it can be applied to simplify the methods of haematological estimations. The economy of time thus obtained will permit an augmentation of the number of individuals in the investigated groups, and as a result from the former, more accurate and precise data was obtained. It might also be interesting to state whether this diagram could be applied to wild carp in rivers („sazan"). How great can the influence of environment be on divergencies of the values discussed here is shown on the data of results obtained in this work and those of the work of Kaname (1954), as listed below:

	Kaname	own results
Specific gravity of blood	1,047	1,030— 1,040
Number of erythr. mil./mm³	2,210	1,150— 1,600
Number of leucoc. thous./mm³	40,2	32,0 —137,6
Haemoglobin content %	35,7	32,5 — 57,0
Haematocrit	0,347	0,309— 0,388
Dimensions of erythr.	10,4 — 15,0 × 7,0 — 9,8 μ	11,2 — 12,8 × 8,2 — 9,2 μ

Unfortunately the Japanese scientist does not explain the exact methods he used for obtaining these values, nor does he note the age of investigated carp. Therefore, it might be incorrect to compare directly these numbers with the results of the present work. It can only be stated, in a general way, that the values given by Kaname are higher than the corresponding ones for the material which I investigated. This fact can be the result of different climatic and breeding conditions of fish in that region. In our conditions, those of a temperate climate, the above diagram shall, I hope, adequately accomplish its task. Separate samples can show differences up to 10% of the investigated value, but when accomplishing a greater amount of determinations in series and having acquired some practice this error can be diminished considerably. I can state for comparison that estimating of the number of erythrocytes by means of the classical method is burdened with an error up to 12%, and when determining the perentage of haemoglobin content by means of Sahli's haematometer one commits an error reaching 10%. That is why I consider the gravimetrical method for determining the specific gravity of blood as worth recommending. It is a substitute for more labourious and not more accurate methods for estimating the number of erythrocytes, percentage of haemoglobin content and haematocrit which are generally in use now.

Conclusions

1. Collecting of 0,1—0,3 ml of blood from carp in their second and third year of life ($K_{1/2}$ and $K_{2/3}$) does not provoke checking of their growth tempo. Losses in individuals of the investigated material, below the generally accepted norm for commercial production, took place both in the groups from which blood was taken and in the control ones. One needs not count therefore with an increase of loss under the influence of the collecting of blood. An increase of sample volume up to 0,6—1,0 ml from one individual induces a 2,4—2,5% decrease of growth in investigated subjects.

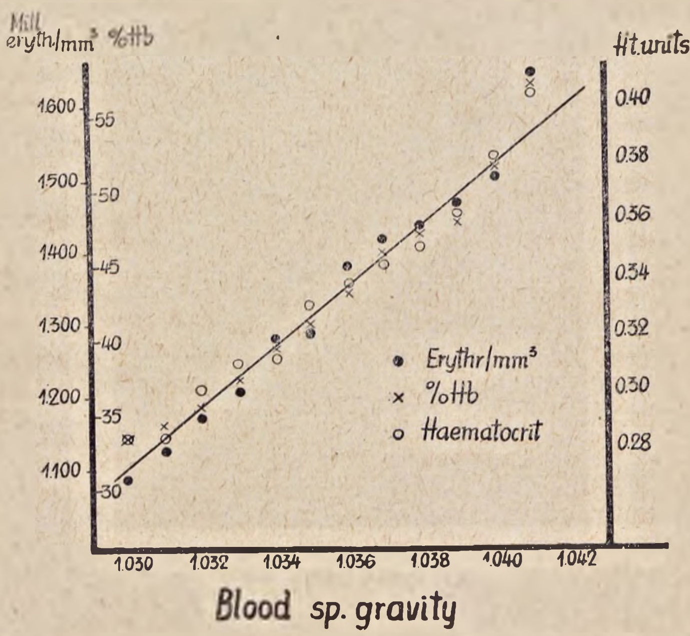

Fig. 4. Diagram for interpolation of erythrocyte number, haemoglobin content, and haematocrit based on the specific gravity of blood values.

2. In the period of 14—28 days from the moment when blood from the fish was collected, a return to the norm is effectuated in the following values: specific gravity of blood, number of erythrocytes in a unit of volume, haemoglobin percentage content, and haematocrit.

3. Collecting of 0,1—0,3 ml blood samples induces a rise of white blood corpuscles in carp for a period of 14—28 days. This could be explained by a defensive reaction of the organism to the operation of blood collecting. It must also be stated that, in general, the number of white blood corpuscles attains its highest value in spring and in autumn.

4. Under the influence of blood collecting erythrocytes assume a more spherical form which would be the proof of a more intensive course of erythropoiesis.

5. Estimations necessitating certain technical equipment and great time expenditure as: the number of erythrocytes for a unit of volume, percentage of haemoglobin content, and the relation of morphotic elements to plasma volume, (haematocrit can be replaced successfully by gravimetrical determination of the specific grafite of blood. When this value is known, it is possible on the basis of the diagram (fig. 4) to interpolate the remaining values.

Lastly, I should like to express my gratitude to all the staff of the Fishery Experimental Station at Mydlniki, and especially to ing. Czubak for the comprehensive help which they gave me in the working out of the problem discussed above.

STRESZCZENIE

Powszechnie utrzymuje się przekonanie, że ryby, od których pobrano krew do badania, nie nadają się do dalszej hodowli, pobranie bowiem krwi osłabia je, opóźniając tym samym ich start w procesie dalszego wzrostu. Praca niniejsza ma na celu wyjaśnienie, czy pobranie krwi od karpi, w drugim i trzecim roku życia w ilości 0,1—0,3 ml od sztuki, odbija się w jakiś sposób na ich tempie wzrostu i późniejszym obrazie krwi. Ponadto z uwagi na dużą pracochłonność klasycznych oznaczeń hematologicznych (liczba erytrocytów na jednostkę objętości, procentowa zawartość hemoglobiny czy stosunek objętości elementów morfotycznych do osocza) oraz związaną z tym konieczność posiadania pewnego wyposażenia technicznego autor proponuje uproszczenie metodyki wyżej wymienionych oznaczeń przez zastąpienie ich grawimetrycznym określaniem ciężaru właściwego krwi, co pozwala na opracowanie większej ilości próbek, w znacznie krótszym okresie czasu, przy jednoczesnej zadowalającej dokładności.

Relacjonowane tutaj badania były wykonywane w Rybackiej Stacji Doświadczalnej w Mydlnikach, należącej do Wyższej Szkoły Rolniczej w Krakowie, na dwu- i trzechletnim karpiu lustrzeniu, w stawach Konrad, Spytek w 1958 roku, oraz Mieszko, Migdał w 1959 roku. Oprócz tego dodatkowo w jesieni 1959 roku przeprowadzone zostały obserwacje na 250 sztukach karpi tego samego wieku z 10 stawów doświadczalnych w kompleksie „Za Młynówką". Na stawach Konrad, Spytek, Mieszko i Migdał ryby znakowane były znaczkami srebrnymi. W 1958 roku do przymocowania znaczków użyta została barwiona nić nylonowa, a w 1959 roku srebrny drut — jak się okazało lepszy do tego celu.

Każdego roku na wiosnę (w maju) zostały wykonane zasadnicze pomiary biometryczne, od połowy zaś sztuk została pobrana krew w ilości 0,1—0,3 ml. Następne pomiary biometryczne całych obsad i pobranie krwi od 20 sztuk z każdego stawu następowało po 14, 28 i 42 dniach od momentu pierwszego pobrania krwi, w połowie lata (koniec lipca — początek sierpnia) oraz w jesieni przy końcowym odłowie (pierwsza połowa października). Każdorazowo brano próbki krwi od 10 ryb, od których już krew była pobierana i dla kontroli od 10 ryb, od których poprzednio jeszcze krwi nie pobierano.

W pobranych próbkach krwi oznaczono: liczbę erytrocytów na jednostkę objętości, procentową zawartość hemoglobiny, liczbę białych ciałek krwi i wymiary erytrocytów mikroskopowo, na barwionych metodą May-Grüwald-Giemsy rozmazach, stosunek objętości elementów morfotycznych do osocza (hematokryt), oraz ciężar właściwy krwi, oznaczamy metodą grawimetryczną. Metoda ta została opracowana dla klinicystyki ludzkiej przez grupę amerykańskich badaczy (Philips, Van Slyke, Hamilton, Dole, Emerson, Archibald 1950a, b). Polega ona na ustaleniu gęstości badanej próbki krwi, względem bardzo dokładnie, z dokładnością do czwartego miejsca dziesiętnego, przyrządzonych standartowych roztworów siarczanu miedzi ($CuSO_4 \cdot 5\,H_2O$). Roztwory takie przy użyciu pyknometru sporządzone zostały dla karpi w przedziale gęstości 1,020 — 1,042 w odstępach co 0,001 lub 0,002. Porcje wynoszące po 50 — 100 ml takich roztworów umieszczone zostały w szkalnych cylindrach o wysokości słupa cieczy 10 cm. (Fig. 1.). Oznaczenie sprowadzało się do wkroplenia po jednej kropli krwi badanej do kilku kolejnych cylindrów. Roztwór standartowy, w którym kropla krwi utrzymała się w połowie słupa rtęci cieczy przez około 10 sekund, wskazywał na gęstość próbki badanej.

W okresie badawczym straty ryb w sztukach przedstawiały się następująco.

1958	staw Konrad	$K_{1/2}$	14,3%
	staw Spytek		0 %
1959	staw Mieszko	$K_{2/3}$	3 %
	staw Migdał		0 %

Otrzymane wyniki odnośnie pomiarów bimetrycznych zestawione są w Tab. III. Wykazuje ona, jak również wykres sporządzony dla średnich ciężarów jednostkowych (Fig. 2), że nawet dwukrotne pobranie krwi od karpi, w drugim i trzecim roku życia, w ilości 0,1 — 0,3 ml od sztuki, nie powoduje zahamowania ich tempa wzrostu. Można nawet zauważyć pewną nieznaczną tendencję zwyżkową w przyrostach sztuk, od których krew była pobierana (Tab. IV). Należy jednak zastrzec się, że zaobserwowane różnice pomiędzy średnimi ciężarami jednostkowymi, opracowane metodą analizy wariancyjnej, okazują się nieistotne (Tab. V).

Jest rzeczą zupełnie zrozumiałą, że przekroczenie pewnej granicy ilości pobranej krwi prowadzi do ujemnych skutków. Pobranie od karpi dwu- i trzechletnich 0,6 — 1,0 ml krwi od sztuki powoduje już pewne zahamowanie ich tempa wzrostu i obniżenie wskaźników hematologicznych (Tab. VI).

Dane liczbowe, dotyczące oznaczeń hematologicznych u ryb ze stawów Konrad, Spytek, Mieszko i Migdał, zestawione są zbiorczo w Tab. VII. Ciężar właściwy krwi, liczba erytrocytów na jednostkę objętości, procentowa zawartość hemoglobiny i hematokryt, w zasadzie już po upływie 14 — 28 dni od momentu pierwszego pobrania krwi, były wyższe u ryb badanych w porównaniu z kontrolnymi, co świadczyłoby, że okres ten jest wystarczający dla organizmu ryby dla uzupełnienia strat spowodowanych pobraniem krwi. W tym okresie daje się również zauważyć silny wzrost liczby białych krwinek (w stawie Mieszko wynosił on 60% wartości wiosennej). W krwi ryb, które dały już wcześniej krew, spotyka się większą ilość kulistych, młodocianych form erytrocytów, co świadczyłoby o wzmożeniu procesu erytropoezy. Powierzchnia pojedynczych erytrocytów nie wykazuje zarówno u $K_1/_2$, jak i u $K_2/_3$ żadnych istotnych zmian pod wpływem pobrania krwi.

Liczba erytrocytów na jednostkę objętości, procentowa zawartość hemoglobiny, hematokryt i ciężar właściwy krwi wykazują wyraźną współzależność zmian (Tab. VIII). Współzależność ta przyjmuje postać funkcji prostoliniowej $y = a + bx$. Na podstawie tej funkcji, w pracy niniejszej obliczone zostały współczynniki regresji **b** przyjmując za zmienną niezależną (x) ciężar właściwy krwi, a za zmienną zależną (y) kolejno pozostałe wyżej wymienione wartości. Obliczenie takie przeprowadzone zostało najpierw dla poszczególnych obsad (Tab. VIII), a następnie dla całego materiału obserwacyjnego (Tab. IX). co pozwoliło, po odpowiednim dobraniu skali, opracować diagram (Fig. 4). Na podstawie tego diagramu, mając dany ciężar właściwy badanej probki krwi, można interpolować liczbę erytrocytów, procentową zawartość hemoglobiny i hematokryt.

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