USE OF THE CORRELATION BETWEEN THE WEIGHT OF EMBRYOS AND THE RELATIVE WEIGHT OF ORGANS TO ASSESS THE ACTION OF SUBSTANCES STIMULATING AND INHIBITING GROWTH OF ORGANS

V. A. Konyshev

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Grafts and extracts of organs, acting upon the embryo, may cause selective stimulation or inhibition of development of its organs. This phenomenon has recently been studied by many investigators, for it may provide the key to the understanding of the mechanisms of organogenesis. Usually such investigations have been carried out on chick embryos. One of the methods which can be used to study the ability of material injected into the ovum to stimulate or inhibit the development of an investigated organ is by determining the size of the organ or its weight after injection of an extract obtained from the tissues [2, 5, 6]. Since the change in the absolute weight of an organ may be the result of a change in the weight of the embryo, investigators have usually determined the relative weight of the organ, the quotient obtained by dividing the weight of the organ by the weight of the embryo. The results are usually presented as histograms, and the question of the significance of the difference between the experimental and control results is reduced to evaluation of the difference between the values of the mean relative weight of the test organ in the experiment and control. This method has been used to study substances stimulating the weight of the liver, spleen, and heart. It is assumed that the values of the relative weight obtained in this manner are independent of the weight of embryos of the same incubation period. However, authors who have used this approach do not cite experimental data proving that in fact no correlation exists between the relative weight of the organs and the weight of the embryos.

If it is assumed that the relative weight of organs changes along with changes in the weight of the embryos, it will be clear that the introduction of this ratio instead of the absolute weight of the organs cannot overcome the difficulties associated with the difference between the mean weight of the embryos of the experimental and control groups or the different distributions of the weight of the embryos by classes in the groups to be compared.

TABLE 1. Correlation between Relative Weight of the Organs of the Embryos

Expt. no.	Duration of incubation (in days)	Number of embryos (n)	Correlation between relative weight of organs and weight of embryos						Correlation between ratio of weight of liver to weight of heart and weight of heart	
			liver		heart		spleen			
			r	P	r	P	r	P	r	P
1	19	7	–0,62	0,850	–0,74	0,942	–0,36	0,540	–0,05	0,158
2	19	13	–0,77	0,998	–0,29	0,657	–0,33	0,719	–0,94	0,999
3	19	27	–0,39	0,956	–0,46	0,986	–0,32	0,905	–0,42	0,972
4	19	14	+0,68	0,993	–0,60	0,977	–0,36	0,769	–0,42	0,838
5	18	10	–0,81	0,999	–0,58	0,962	–0,03	0,071	–0,71	0,994
6	18	10	–0,04	0,095	–0,67	0,989	–0,77	0,998	–0,45	0.866

Legend: r – coefficient of correlation; P – probability of the existence of correlation found on the basis of the criterion $K = 0.5\,[\ln(1+r) - \ln(1-r)]\sqrt{n-3}$ from tables of values of the integral of probabilities [3].

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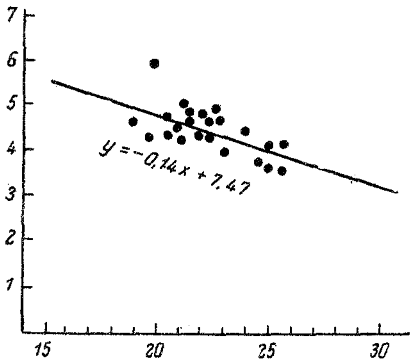

Fig. 1

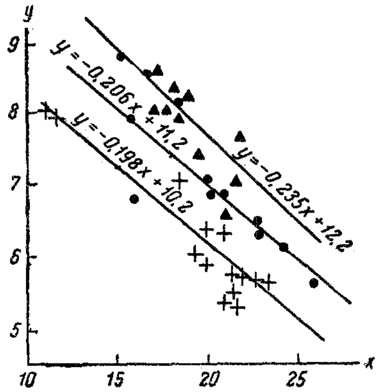

Fig. 2

Fig. 1. Relationship between relative weight of the heart (y) and weight of the embryos (x). Coefficient of correlation – 0.46 ± 0.11. Here and in Fig. 2: along the axis of ordinates – relative weight of the heart ($\times 10^3$), along the axis of abscissas – weight of embryos (in g).

Fig. 2. Effect of injection of liver ribonucleoprotein into the ovum on the relative weight of the embryos' heart. Crosses – control (injection of 0.85% NaCl solution); circles – ribonucleoprotein not treated by chloroform (ratio between extinctions during spectrophotometry at wavelength of 260 and 280 mμ ($E_{260}/E_{280} = 1.61$); triangles – ribonucleoprotein after treatment with chloroform ($E_{260}/E_{280} = 1.77$).

In the present investigation an attempt was made to discover whether a correlation exists between the relative weight of the liver, heart, and spleen and the weight of the embryos.

EXPERIMENTAL METHOD

Experiments were carried out on chick embryos of the Russian White breed of 18 and 19 days of incubation. Embryos of this age are used for assessing the action of substances regulating the growth of organs. The embryos, freed from the membranes, were weighed on apothecaries' scales and the organs (liver, heart, and spleen), on ADV-200 analytic scales. To avoid drying of the organs they were weighed in weighing bottles beneath a layer of mineral oil. The relative weight of the organs was calculated from the results obtained. By using the methods of correlation analysis [3] the coefficients of correlation between the weight of the embryos and the relative weight of the heart, liver, and spleen were calculated.

The correlation was also studied between the ratio of the weight of the liver and heart and the weight of the heart. To obtain a clear picture of the results, histograms were not used, as is usually done by workers studying the action of substances regulating growth, but graphs, where the weight of the embryos was plotted along the abscissa and the relative weight of the organs along the ordinate. Altogether 6 experiments were carried out on 81 embryos.*

EXPERIMENTAL RESULTS

The experimental results are given in Table 1, showing the coefficients of correlation between the relative weight of the organs (liver, heart, and spleen) and the weight of the embryos. The coefficients of correlation between the relative weight of the liver, taking the weight of the heart as unity, and the weight of the heart are also shown in Table 1.

The results obtained show that at the incubation times studied no correlation was present between the relative weight of the organs and the weight of the embryo (r close to 0) in a few cases. In the overwhelming majority of cases an inverse relationship was present between these values (r was expressed by negative values, differing from 0) or there was a clearly defined tendency toward such a relationship (Fig. 1)

* The experimental part of the investigation was carried out with the help of Senior Laboratory Assistant L. V. List.

Hence, in most of the experiments, with an increase in the weight of the embryo, the relative weight of its organs diminishes. A similar relationship was observed if the relative weight of the liver was calculated by dividing its absolute weight by the weight of the heart. It is clear from these results that the difference between the mean weight of the embryos in the experimental and control groups and the unequal distribution of the values of the weight of the experimental and control embryos among classes may be accompanied by differences in the relative weight of the organs in the embryos of the groups. This may be the reason for the mistaken conclusion that a substance injected into test embryos possesses an organo-specific effect.

In connection with the facts described above, it is obvious that the changes in the relative weight of organs can be analyzed only by taking into account the correlation links between the weight of the organs and the weight of the embryos. The most accurate method of assessing differences in the weight of organs in compared groups of embryos are those combining correlation and dispersion analysis [3, 4].

For instance, when the effect of liver ribonucleoprotein (before and after partial deproteinization with chloroform by Cohen's method [7]) was investigated, the results given in Fig. 2 were obtained. From these results the hypothesis was put forward that the relative weight of the heart increased in this experiment under the influence of the investigated fractions. Application of dispersion analysis [3] provides a means of assessing the probability of the hypothesis that the experimental lines exceed the control. In this particular experiment this hypothesis was confirmed ($P < 0.01$). The most probable values of the coefficient in the equations of the investigated lines may be found by using the method of least squares [1] (Figs. 1 and 2). However, in mass experiments the need for this does not arise because the equations of the regression lines are not used during statistical analysis of the material.

It is clear from Table 1 that in some of the experiments no correlation was found between the weight of the embryos and the relative weight of the organ. In these experiments the weight of the embryos did not vary enough for a clear correlation to be found between the weight of the organs and the weight of the embryos. However, methods of analysis of the experimental data should be chosen on the basis of the general principle, i.e., the presence of correlation.

Hence, during analysis of the relative weight of the organs of embryos its correlation with the weight of the embryos themselves must be taken into account, for the introduction of the relative weight instead of absolute does not remove the correlation between the weight of the organ and embryos. Evidently, the traditional analysis of the relative weight of organs, supplemented by the study of its correlation with the weight of the embryos, has no essential advantage over analysis of the relationship between the absolute weight of the organ and the weight of the embryo. It is therefore absolutely in order, without calculating the relative weight, to investigate the relationship between the absolute weight of organs and of the embryo using the methods described [3, 4, etc.].

The effect of test substances on the weight of the organs of embryos may be investigated most fully by means of two-factor dispersion analysis [4]. This method can be used to establish the reliability of the effect of the test substance (factor A) on the weight of an organ and to assess the degree of an organ and to assess the degree of its statistical effect, the reliability and degree of the influence of the weight of the embryo (factor B) on the weight of the organ, and the reliability and degree of the effect of the injected substance depending on the gradation of factor B, i.e., to answer the question whether the effect of the injected substance depends on the weight of the embryo (in other words, to investigate the combination of action of factors A and B).* Since embryos of different weights in the experimental and control groups cannot be distributed beforehand by classes by arranging the experiment in a special way, the dispersion complexes obtained are usually unequal and they can be analyzed only by the schemes for analysis of unequal complexes. An example of such a complex is shown in Table 2, presenting the results of one experiment to study the effect of an extract of spleen, freed from ribonucleoproteins, on the weight of the embryos' liver. From the data available, a two-factor complex was formed, in which one factor (A) was the action of the test extract (with the action of physiological saline as the control) and the other factor (B) was the gradation of weight of the embryos. The results were analyzed by the third scheme for the analysis of unequal dispersion complexes [4].

As is clear from Table 2, injection of the test extract had an inhibitory action on the growth of the liver ($F_A = 12.2$; $P < 0.01$), and the degree of the statistical effect of the extract on the weight of the liver (η^2_A) was 0.34. Factor B (the weight of the embryos) in this experiment did not have a strong, statistically significant effect

*This method assumes the mutual independence of the investigated factors, and its use is limited to experiments in which the test substance has no effect on the weight of the embryos.

TABLE 2. Use of the Third System of Analysis of Unequal Dispersion Complexes when Investigating the Effect of an Extract of Spleen, Freed from Ribonucleoprotein and Treated by Dialysis, on the Weight of Embryonic Livers. Two-factor complex

A_1 Treatment with extract		A_2 Treatment with 0.85% NaCl	
B_1 17—18	B_2 19—21	B_1 17—18	B_2 19—21
weight of liver			
420 412 408 405 448 423	410 437 512 470 424	506 444 505 492 503 424	509 467 483 447 452 492 478
419	451	481	475

Note. Weight of embryos (B) in grams, weight of liver (A) in milligrams.

Results of analysis of complex

	A	B	AB	X	Z	U
C	11,013	1,299	2,122	14,433	17,033	32,366
η^2	0,340	0,041	0,066	0,447	0,553	1,00
V	1	1	1	3	20	23
σ^2	11,013	1,299	2,122	4,811	898	—
F	12,2	1,45	2,36	5,35	—	—

Value of confidence limits (F)

V_2	V_1 1	2	3	P
20	14,8 8,1 4,3	10,0 5,8 3,5	6,5 4,1 2,7	0,001 0,01 0,05

on the weight of the liver ($\eta^2_B = 0.041$, $F_B = 1.45$; $P < 0.05$). The combination of the action of factors A and B resulted in $\eta^2_{AB} = 0.066$ ($F_{AB} = 2.36$; $P < 0.05$), and the over-all effect of factors A, B, and AB — $\eta^2_X = 0.447$ ($F_X = 5.35$; $P < 0.01$). The citation of the results of individual experiments in this paper is intended only to illustrate the essence of the problem, without at all trying to make far-reaching conclusions concerning the character of action of the test fractions on the basis of the results of individual experiments. In fact, having obtained in one of the experiments $P \approx 0.01$, it may be expected that another absolutely identical fraction in an experiment on absolutely identical embryos would give the same effect, but in order to generalize this conclusion, the experiment must be repeated on several successively obtained fractions and new batches of embryos.

When the methods of dispersion analysis are used, experimental and control points must be present in each of the classes into which the variant is subdivided. In some experiments it happens that in one of the groups to be compared the extreme values of the weight of the embryos lie outside the limit of the extreme values of the weight of the other groups of embryos. These variants cannot be taken into account when forming the complex. Only if the experimental and control lines run parallel, which can be seen from the equations obtained by the method of least squares, can the distance between the lines and the dispersion (or the standard errors) of the points be calculated and the significance of the difference between the compared lines be determined, using dispersion analysis or Student's method.

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