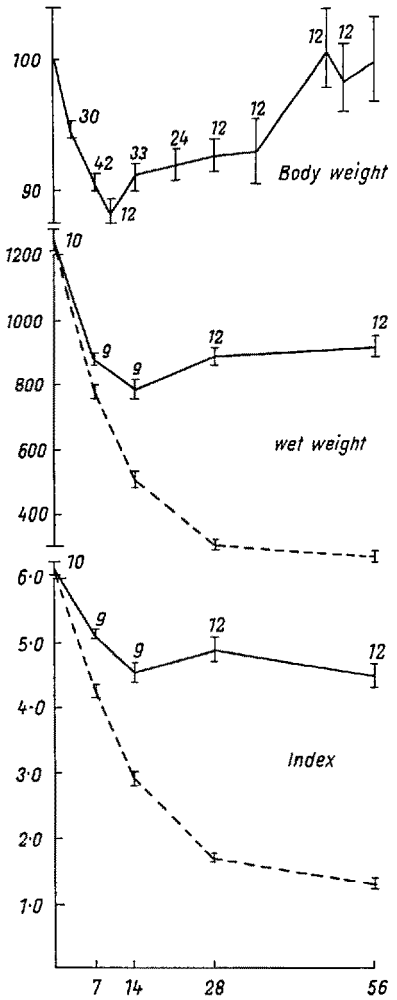
there is a distinct loss of body weight during the first 7–9 days which is later followed by a gain in body weight, so that 56 days after the operation the initial body weight is regained.

It was found that during the first phase, the atrophy on the plegic side progressed at the same rate as that of the denervated muscles (Figure). No differences were noted between the wet and dry muscle weights.

Following this, there was a gradual but significant gain in weight in both the gastrocnemius and soleus 56 days after transection of the cord, as compared with their lowest values. These results are thus in accord with the findings of FISCHER[1], SOLANDT and MAGLADERY[2].

However, the evaluation of results in this type of experiment in absolute units only (mg) does not take into account the changes due to body weight which can influence the muscle weights. In order to elucidate the role played by the temporary loss of body weight in the above changes, all the muscle weights were calculated as an index: $\frac{\text{muscle weight in mg}}{\text{body weight in g}}$. It was found that during the first phase following section of the spinal cord, atrophy of the plegic muscles does actually occur, i.e. that the muscles lose weight at a faster rate than would correspond to the loss in body weight. But during the second part of the experiment, the index values do not exhibit a tendency to increase, i.e. the absolute gain in weight of the soleus and gastrocnemius is proportional to the gain in body weight (Figure).



The course of changes in body weight, muscle wet weight, and index of muscle to body weight in rats, 7, 14, 28, and 56 days after complete transection of the spinal cord and unilateral section of the sciatic nerve. Body weight expressed as percentage of initial values, muscle wet weight given in mg. Continuous line: lower motor neuron intact; dotted line: lower motor neuron transected. Vertical lines represent standard error of the mean, numerals give number of animals in each group. Abscissa: time after operation in days

It is thus possible to explain the restitution of weight of the gastrocnemius and soleus, i.e. muscles investigated by FISCHER, SOLANDT, and MAGLADERY, in the first place by the increase of body weight of the experimental animals.

P. HNÍK

Physiological Institute, Czechoslovak Academy of Sciences, Prague, August 26, 1959.

Zusammenfassung

Nach Myelotomie verläuft die Muskelatrophie in zwei Phasen. In der ersten Phase stimmt der Gewichtsverlust der Muskeln mit demjenigen nach Denervation überein. Dagegen erfolgt in der zweiten eine Gewichtszunahme, die durch die analoge Gesamtzunahme des Körpergewichts erklärt wird.

PRO EXPERIMENTIS

A Method of Determining Electrophoretic Mobilities of Antibodies

The localization of antibody activity after electrophoresis has been determined by several methods. It has been done using free electrophoresis[1], paper electrophoresis[2,3], and agar gel electrophoresis[4]. In the latter cases[2–4] the antibody activity has been determined in fractions of the separated serum obtained by elution from the paper or the agar after the electrophoretic run. A method has also been published, however, with which hemagglutinins can be localized after paper electrophoresis by the disposal of red blood cells directly on the paper after the electrophoretic run[5]. This method – although convenient – has been reported not to be very sensitive and does not seem to allow a very precise localization of the antibodies[6]. The present report discusses a technique in which antibodies can be accurately localized by antigen-antibody reactions in basins placed in direct contact with the electro-separated material after the run.

Technique: The electrophoretic separation is performed in agar as described by WADSWORTH and HANSON[7]. Buffered agar of 1% is poured on a 8·2 × 8·2 cm photographic glass to which is attached by adhesive tape two glass pieces 2 × 8·2 cm on opposing sides. The material to be analyzed is mixed with agar and placed in 3 mm wide cut basins. After the separation is complete, the glass end pieces, which provide contact with the buffer in the electrode vessels, are removed. A matrix (78 × 15 mm) of 3 mm thick plexiglass, in which basins 3 mm in diameter are formed, is placed on top of the agar over the separated material (Fig.). The basins in the matrix are placed so that they cover areas which overlap each other along the axis of separation. It is important to eliminate confluence of the material between these different basins. Therefore the matrix is provided with a piece of 0·5 mm thick waterproof tape at each end and melted agar of

[1] J. R. CANN, R. A. BROWN, J. G. KIRKWOOD, P. STURGEON, and D. W. CLARKE, J. Immunol. *68*, 243 (1952).

[2] B. D. JANKOVIC and H. W. KRIJNEN, Nature *171*, 982 (1953).

[3] R. PAYNE and Q. B. DEMING, J. Immunol. *73*, 81 (1954).

[4] R. FAURE, J.-M. FINE, M. SAINT-PAUL, A. EYQUEM, and P. GRABAR, Bull. Soc. Chim. biol. *37*, 783 (1955).

[5] G. BERG, W. FRENGER, and F. SCHEIFFARTH, Klin. Wschr. *33*, 767 (1955).

[6] A. SEMLOW, Z. Immun-forsch. *116*, 215 (1958).

[7] C. WADSWORTH and L. Å. HANSON, Int. Arch. Allergy, in press (1960).

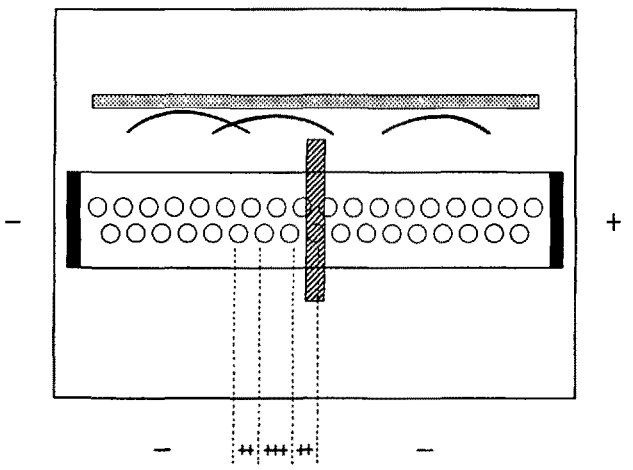
0·5% is poured between the matrix and the agar layer to seal the basins. After the electrophoresis, these basins are about 1/3 filled with saline. The plate is then kept at 37°C for 30 min. To determine hemagglutinating antibodies the basins are then filled up with a 2% suspension of red cells antigenically corresponding to the antibodies to be demonstrated. After an additional period of time (20–60 min) at 37°C, the material in each basin is well mixed with a capillary pipette and the cell suspension is removed. A microscopic examination for agglutination is made. To determine the localization of the serum fractions after the electrophoretic separation, immunoprecipitation is performed. This is accomplished by application of the corresponding immune serum in a basin (2 × 70 mm) cut parallel to the direction of the electroseparation and 4–6 mm from the starting basin (Fig.). The plate is then kept in a humid atmosphere at room temperature, and the next day, after the immune electrophoretic spectrum has developed, a photograph is taken before the matrix is removed.

Experimental: Control experiments were performed in order to determine if diffusion in the agar after the electrophoretic separation changed the localization of the substances to be detected. It was found that unseparated serum-agar containing the isoagglutinin anti-A showed strong agglutination of A-cells in the matrix basin which was situated directly above the serum agar basin. One basin on each side of this showed weak reactions, but each of these covered 1 mm of the serum basin. No agglutination was observed in the basins which were 1 mm from the serum basin even after several hours.

Using the method described, agglutinating antibodies could be electrophoretically localized with great accuracy. Separating human sera containing the isoagglutinins anti-A and anti-B, these antibodies could be localized in the β_1- and β_2-globulin regions as seen in the Table. Agglutinating anti-Rh antibodies (anti-D) were mainly seen in the β_1- and somewhat out in the β_2-globulin region when using 0 Rh(+) red cells in saline, but the agglutination was extended to both sides especially into the γ-globulin region, when papain-treated cells were used instead (Table). Incomplete anti-D antibodies could be demonstrated in the β_1-globulin region when 20% human albumin was added to the matrix basins instead of the saline. Using papainized cells agglutination was noted also in the β_2- and γ-globulin regions.

Antibodies could also be localized in material other than blood serum using this technique. Thus anti-A and anti-B isoagglutinins were found in colostrum in the area corresponding to the localization of the β_1- and β_2-globulins of serum. A more detailed report on these findings will follow[8].

Results were also obtained which indicated the possibility of localizing the presence of antibody activity other than agglutination. A serum with a high anti-streptolysin 0 titer was electro-separated, reduced streptolysin 0 was added to the basins in the plexiglass matrix, and after 30 min these were filled up with a 2% sheep red blood cell



Schematic drawing of an analysis detecting the electrophoretic mobility of antigens or antibodies The material to be separated is put in the central basin and the plexiglass matrix is placed on top of the agar, covering the separated material. The matrix basins are 3 mm wide and the distance between the center of two adjacent basins in one row is 4 mm. This distance forms the base of an isosceles triangle with 5 mm sides. A basin in the other row has its center in the third corner of this triangle. The immune serum corresponding to the separated material is filled in the longitudinal basin. The activity found (indicated by +) is localized by means of the immune electrophoretic spectrum

[8] L. Å. HANSON, in manuscript.
[9] L. GORECZKY und N. MIKLÓS, Z. Immun-forsch. *118*, 490 (1959).

Electrophoretic Localization of Antibodies

Serum Specimen	Corresponding Immune Reactant	Antibody Activity
		Hemagglutination
anti-A	A Rh(–)cellls in saline	
anti-B	B Rh(-)cells in saline	
anti-Rh (D) aggl.	0 Rh(+) cells in saline	
anti-Rh (D) aggl.	0 Rh(+) papain-treated cells	
		anti-Hemolysis
anti-streptolysin 0	Reduced Streptolysin 0 (sheep cells as indicator)	
Serum fractions as localized by immune electrophoresis		γ β_2 β_1 α_1 α_2 alb.

suspension. The hemolytic activity of the lysin had been neutralized in the basins above the β_2-globulin region where the anti-streptolysin 0 antibodies were evidently localized after the electrophoresis (see Table).

It also seemed possible to localize hemolysins after electroseparation of a streptococcal culture filtrate by means of their lytic activity on red blood cells. The hemolysis could easily be read directly in the basins.

This method thus seems to allow an accurate determination of the localization of different antibody and toxin activity after electrophoresis. However, further work may be needed to develop the technique and to determine factors of importance for the results obtained; e. g. the amount of material separated has been reported to influence the distribution of activity determined[3,9].

The human red cells and sera were kindly supplied by Dr. L. RYTTINGER, The Blood Bank, Sahlgren Hospital, Gothenburg.

L. Å. HANSON, V. RAUNIO, and C. WADSWORTH

Department of Bacteriology, University of Gothenburg (Sweden) and Department of Microbiology, University of Turku (Finland), December 30, 1959.

Résumé

On expose une méthode simple par laquelle il a été possible de localiser exactement l'activité des anticorps ou des antigènes après électrophorèse. Cette méthode a été appliquée aux hémagglutinines du sérum et du lait, aux antitoxines et hémolysines.

STUDIORUM PROGRESSUS

Methods of Obtaining and Mode of Action of Stage Ontogenins

By G. K. ROUSSEV[1]

In studying the connections between the preformed and epigenetical state of individual development, the necessity of applying new methods for this purpose[2] arises more and more.

Recently a number of authors have succeeded in producing specific disturbances at a clearly defined stage of embryonic development by making use of antimetabolites of nucleoproteid metabolism[3,4].

In 1954, I found a new biological method of obtaining bioactive substances influencing embryonic development[5]. I observed that, after conglutination of father-spermazozoa to the naked surface of the developing embryo of *Triturus cristatus*, the surrounding solution acquires new qualities. This solution stops the development of younger embryos submerged in it and at that same stage of the naked embryo; older embryos develop unhindered[6]. We supposed, that the action of the solution is due to the formation of substances which were named *stage ontogenins*, bearing a number corresponding to the number of the stage of the embryo from which they are obtained[7].

Together with G. NAIDENOVA, experiments were performed to purify the native solutions of some ontogenins – 3, 4, 9, 10. Applying different biochemical methods for isolation, it was found that the action is due to the presence of strictly defined chemical substances[8] – for example by chromatogram and UV-absorption curve, the presence of phosphorus in the ester-bond of purified ontogenin 4 indicates that the latter is structurally similar to a deoxyribosenucleotid.

We had to get more data of the mode of action of the ontogenin.

Method. We worked with *Triturus cristatus* during its period of heat. The animals were caught at their natural breeding grounds and within 36 h the eggs were artificially fertilized by Hertwig's method, keeping instruments and materials sterile. The spermatozoa, both for fertilization and later for conglutination, were obtained by massaging the abdomen to cause an ejaculation which was afterwards diluted in Holtfretter solution. The experiments were carried out in small porcelain crucibles with waxed bottoms. The crucibles were placed in a gas chamber in an oxygen atmosphere at 100% relative humidity and 17–19°C.

The gelatinous envelope and the viteline membrane of the embryos, at different stages of development were removed in such a way as to cause a slight injury. The naked embryo in this way was rinsed for 10–15 min in already used triton blood serum and transferred to a 1·5 ml Holtfretter solution containing $1^0/_{00}$ glucose. This solution contained in suspension about 50000 spermatozoa recently ejaculated by the father-triton. The spermatozoa rapidly conglutinated to the surface of the naked embryo, especially to the injured parts. Usually after 4–8 h, the embryo was taken away and the solution was decanted and filtered.

The necessity of preservation of these conditions was proved in a series of experiments in which we changed some of the components and kept the rest constant. For ontogenins to be formed, it is necessary for the descending embryo to be without gelatinous envelope and vitelline membrane. If the envelopes are not removed, the spermatozoa cannot penetrate through them and we cannot establish any changes in the surrounding solution. This gave us the possibility to place uninjured embryos with the naked or injured ones – the development of the latter served us as a quick test to estimate the action of the ontogenin obtained by the first. It was usual that in one test two uninjured embryos, one young and the other at an older stage, were placed in the solutions together with the injured one.

It was found that the state of the naked embryo is of the greatest importance and that ontogenins are separated only at definite moments of the different stages and states of these embryos. For example, while maintaining all the conditions for obtaining ontogenins, we obtained ontogenin 4 out of 20 embryos in the stage 10 min after the beginning of the second cleavage till the moment of complete normal second cleavage. 40 naked embryos, 30 and 60 min after the second cleavage did not give ontogenins. Stage 2 gave ontogenins: one 25–45 min after fertilization, one $1^1/_2$–2 h, and one 6 h after fertilization. The embryos at stage 4 h after fertilization are inactive. Only in the beginning of the first cleavage Ontogenin 3 is separated. Stage 10, at the beginning of the gastrulation, and stage 12 at the moment, when the blastopore is closed to a slit, did not give ontogenins.

[1] Medical Research Institute Sofia and Institute of Morphology at the Bulgarian Academy of Science.

[2] C. H. WADDINGTON, *Principles of Embryology* (Allen and Unwin, London 1956).

[3] K. B. LIEDKE, M. ENGELMANN, and S. GRAF, J. exper. Zool. *127*, 201 (1954).

[4] S. BIEBER, J. cell. comp. Physiol. *44*, 11 (1954).

[5] G. K. ROUSSEV, C. R. Acad. bulg. Sci. *4*, 94 (1956) (englisch).

[6] G. K. ROUSSEV, Bull. sect. sci. biol. med. Acad. Sci. Bulg. *1*, 88 (1957) (bulgarisch).

[7] A. POLISTER and H. MOORE, Anat. Rec. *68*, 489 (1937).

[8] G. K. ROUSSEV, Prov. 4th Int. Congress Biochemistry Symp. VI-Bioch. of Morphogenesis (Disc.), in press (1958).