from the molarity at 50% inhibition. The reaction with the homologous antigen DNP-BSA was set one. DNP-BSA was labelled with the iodine monochloride method of MCFARLANE[13].

Results. By using DNP-BSA as immunoadsorbent and DNP-BGG as immunogen, only DNP-specific but no carrier specific antibodies were isolated. This antibody population precipitated DNP-BSA as well as DNP-BGG. The average intrinsic association constant (K_0) with DNP-L-lysine was 2.0×10^5. Precipitin reactions were also observed with 5-acetyluracil-BSA and with purine-6-oyl-BSA. By means of immunoadsorbents consisting of 5-acetyluracil-BSA and purine-6-oyl-BSA, it was possible to isolated the fractions cross-reacting with these two hapten-protein-conjugates. The corresponding yields were about 20 to 25% for acetyluracil-BSA and between 15 and 20% for purine-6-oyl-BSA. The two cross-reacting subfractions were compared with the whole anti-DNP population by isoelectrofocusing in polyacrylamide gel, using a pH gradient from 3 to 10. (Figure). As may be seen from the Figure, no significant differences between the whole anti-DNP antibody population and the fractions eluted from the 5-acetyluracil-BSA or the purine-6-oyl-BSA immunoadsorbent respectively were detectable in the isoelectrofocusing pattern, indicating that the isolation of these subpopulations does not lead to a discernible decrease in heterogeneity.

Binding data of the whole anti-DNP antibody population for various hapten-protein-conjugates are given in the Table. As expected, the highest affinity was observed with the homologous antigen DNP. Similar results were obtained with rabbit anti-DNP antibodies (with a K_0 value of 3×10^7), indicating that the obtained results are not species specific.

When the antibody population obtained after immunoadsorption of the fraction crossreacting with 5-acetyluracil-BSA was studied, the same interactions could still be observed. The relative affinity for 5-acetyluracil-BSA, however, was now about 3 orders of magnitude smaller; the relative affinities for the other hapten-protein-conjugates were also decreased but to a lesser extent. The affinity for DNP-L-lysine of this fraction did not differ from that of the whole anti-DNP population.

Discussion. These results indicate that essentially every single anti-DNP antibody shows multiple binding functions with various hapten-protein-conjugates, but that the relative affinities towards these compounds differ within certain subfractions of the whole anti-DNP population.

The relative affinities against the cross-reacting compounds seem to be at least two or more orders of magnitude smaller than for the immunizing antigen. These differences in affinity would still be compatible with a rather high specificity of an individual antibody molecule. Moreover one could argue that the observed reactions only occur in vitro. VARGA et al.[14], however, were able to demonstrate that 2 structurally dissimilar haptens coupled to a carrier may stimulate the production of a immunoglobulin binding both haptens, presumably by activation of the same cell-surface receptor. This suggests that these multiple binding reactions do not only occur in vitro, but that, inspite of the differences in the relative affinities, they play a significant role in the stimulation and probably also the maturation of antibodies. Moreover the existence of polyfunctional regions within the antibody combining site would have the consequence that fewer antibody species would be required since one antibody molecule might bind several structurally different antigens.

On the other hand, it remains to be proved whether the results reported for antibodies elicited by the DNP determinant are also valid for other antigen-antibody systems.

Binding inhibition data

Compound	Concentration at 50% inhibition (M/l)	K (rel.)
2,4-Dinitrophenyl-BSA	3×10^{-10}	1
Nitroazidophenyl-BSA	9×10^{-8}	3.3×10^{-3}
5-Acetyluracil-1-BSA	1.5×10^{-7}	2.0×10^{-3}
Guanosine-BSA	6.5×10^{-7}	4.8×10^{-4}
Purine-6-Oyl-BSA	5.5×10^{-6}	5.5×10^{-5}
Uridine-5′-Monophosphate-BSA	4.0×10^{-5}	7.7×10^{-6}
Adenosine-5′-Monophosphate-BSA	$> 10^{-4}$	
Cytidine-BSA	$> 10^{-4}$	
p-Azobenzenearsenate-BSA	$> 10^{-4}$	
Dansyl-BSA	$> 10^{-4}$	
BSA	$> 10^{-4}$	
RNA	$> 10^{-4}$	
DNA native	> 1 mg/ml	
DNA single stranded	> 1 mg/ml	

Zusammenfassung. Spezifitätsuntersuchungen an anti-Dinitrophenyl-Antikörpern unter Verwendung einer Methode, die eine Bestimmung der relativen Assoziationskonstanten gegenüber verschiedenen Hapten-Protein-Konjugaten gestattet.

W. RIESEN and A. MORELL

Institute for Clinical and Experimental Cancer Research, University of Berne, Tiefenau-Hospital, CH-3004 Bern (Switzerland),
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Automatic Assay of the Distribution of ^{3}H-, ^{14}C- and ^{32}P-Labelled Compounds within an Early Chick Embryo by a Semiconductor Detector

Autoradiography[1] has hitherto been used as a single non-destructive assay of the distribution of β-nuclide labelled substances in embryology. The main advantage of the semiconductographic method[2], using a computer-controlled device with a special silicon barrier detector[3], consists in a quantitative determination of the particular β-nuclides in the presence of each other at each location of the differentially labelled sample. Moreover, records may

Labelled substances applied

Substance	Specific activity	Amount in single dose (μCi)	Main metabolic fate
^{32}P-Orthophosphate	8.8 mCi/ml	0.5	Incorporated in NA
^{14}C-Orotic acid	164 mCi/m*M*	0.5	Incorporated in NA
^{3}H-Thymidine	6 Ci/m*M*	4.0	Incorporated in DNA
^{14}C-L-Leucine	90 mCi/m*M*	0.5	Incorporated in proteins
^{3}H-6-Azauridine	19.6 Ci/m*M*	4.0	Inhibitor of de novo pyrimidine biosynthesis

be obtained by a fully automated process, the biological material tested does not require any previous processing and the activity is determined in an objective manner.

The detector used makes possible a sufficiently sensitive detection of β-tracers including tritium at atmospheric pressure and room temperature. Up to the present time, it has been possible in tracer experiments to use silicon barrier detectors only in estimations of the β-nuclides with a higher energy spectrum (e.g., ^{32}P, ^{45}Ca, ^{35}S)[4-6]. The use of diffused silicon detectors[7] for tritium has been extremely limited by the depth of the entrance window. By the introduction of semiconductography into experimental embryology, we wish to offer a method which would make it possible to determine the time and spatial patterning of metabolic parameters during normal as well as pathological development at the level of an embryo and morphogenetic systems of embryonic components.

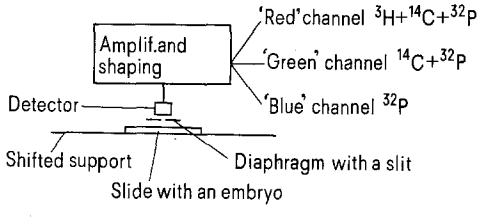

Fig. 1. Functional diagram of semiconductographic device for simultaneous determination of ^{3}H-, ^{14}C-, and ^{32}P-labelled substances distribution.

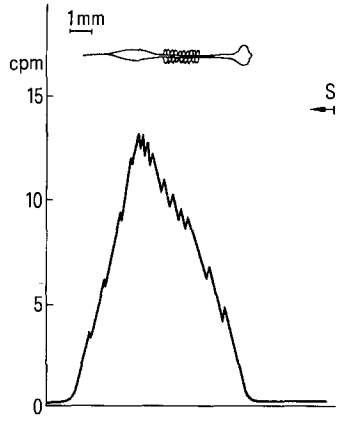

Fig. 2. Semiconductographic record of ^{3}H-thymidine distribution in chick embryo at the HH 10 stage (36 h of incubation). S, reference point. The measured counting rates refer to concrete embryonal structures, making it possible to determine distribution of the labelled substance in the longitudinal axis of the embryo.

Materials and methods. The eggs of White Leghorns were incubated for 36 h. Substances labelled with ^{3}H, ^{14}C, and ^{32}P (Table) were injected into the subgerminal cavity; the total volume of a single injected dose did not exceed 10 μl. The closed eggs were returned into the incubator. After an elapse of 60 min, the embryos were withdrawn, thoroughly rinsed, and kept in air at room temperature on a slide until dry.

The slide with the sample was continuously moved in the craniocaudal direction at the rate of 20 mm/h below a 0.1 mm high golden diaphragm equipped with a slit perpendicular to the moving direction (Figure 1); slit width, 0.5 mm, distance of the diaphragm from slide, 0.3 mm. In the distance of 0.1 mm above the diaphragm, there was placed a detector. In the simultaneous determination of ^{3}H, ^{14}C, and ^{32}P, the resolution of radionuclides was accomplished by an amplitude analysis in three counting channels. Discrimination levels of channels were adjusted according to the energy range of emitted spectra and according to the noise of the counting device, namely, from 9 to 18 keV (the 'red' channel for ^{3}H + ^{14}C + ^{32}P; background, 0.09 ± 0.02 cpm), from 22 to 156 keV (the 'green' channel for ^{14}C and ^{32}P; background 0.17 ± 0.02 cpm), and from 160 to 250 keV (the 'blue' channel for ^{32}P alone; background, 0.11 ± 0.02 cpm). The proportion of particular nuclides was determined analogously to measurements on the liquid scintillation spectrometer[8]. In the case of simple labelling, the lower discrimination level is defined by the requirement to exclude noise from the record (9 keV) while the upper level is determined by the maximum energy of the β-spectrum (^{3}H and ^{14}C) or by the depleted layer (^{32}P). The record of counting rates was performed via an integrator and chartwriter moving at the rate of 120 mm/h.

For the evaluation of records, reference marks were put on the recording paper and each sample. The contours of the embryo and the principal embryonic structures were drawn under the drawing device (Wild) and the counting

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rate curve of the particular radionuclide was correspondingly enlarged in relation to the moving rates of the recording paper and the sample (Figure 2). For each substance or combination of substances, there was evaluated a group of 4–6 embryos applied at HH 9–11 stages. Multiplication of the average peak counting rate by the peak abundance in the corresponding location and allotment of the values thus obtained to the embryo drawing afforded an illustration of the distribution of labelled substances in these stages (Figure 3). Differences due to self-absorption of tritium were neglected since in the central nervous system the cellular density on the craniocaudal axis is almost constant at the stages employed. To verify the absence of any contamination, we have determined the activity distribution after turning the slide with the embryo by 90°; after this turning, the activity occurred in the sample only

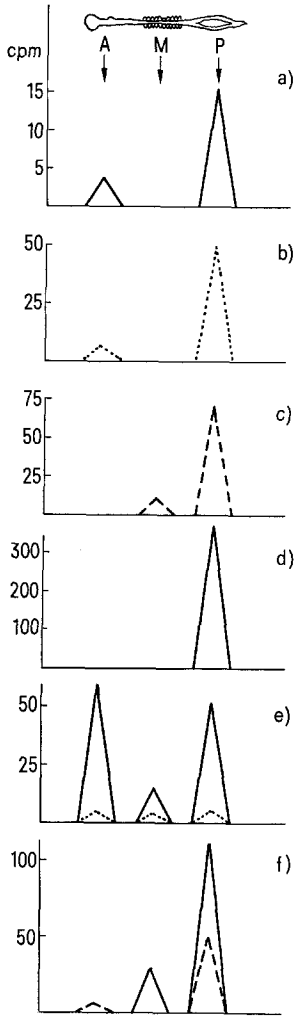

Fig. 3. Mean distribution of labelled substances after the application in developmental stages HH 9–11. a) 4 μCi ^{3}H-thymidine; b) 0.5 μCi ^{14}C-orotic acid; c) 0.5 μCi ^{14}-leucine; d) 0.5 μCi ^{32}P-orthophosphate; e) 4 μCi ^{3}H-6-azauridine + 0.5 μCi ^{14}C-orotic acid + 0.5 μCi ^{32}P-orthophosphate; f) 4 μCi ^{3}H-6-azauridine + 0.5 μCi ^{14}C-leucine + 0.5 μCi ^{23}P-orthophosphate. A, M, P indicate position of the peaks in the anterior, middle and posterior regions of the embryo.

Results and discussion. The main peak of ^{3}H-thymidine, ^{14}C-orotic acid, ^{4}C-leucine, and ^{32}P-orthophosphate was found above the caudal morphogenetic system of the embryo (Figure 3, a–d) which in these developmental stages represents the principal morphogenetic centre[9]. The considerable lower secondary peaks are located above the head and the heart region in the case of ^{14}C-orotic acid or ^{3}H-thymidine, and in the case of ^{14}C-leucine, above the differentiating somites and the neural tube in the middle part of the trunk. When ^{3}H-6-azauridine was simultaneously applied in the teratogenic dose (30 μg), remarkable disturbance of the ^{14}C-orotic acid, ^{32}P-orthophosphate, and ^{14}C-leucine distribution was observed, the ^{3}H-6-azauridine being not incorporated at all (Figure 3e and f). The disturbance involved not only lowering of the peaks but also changes in their pattern with respect to that obtained in the absence of the teratogen. The samples did not exhibit any activity when 4 μCi of ^{3}H-6-azauridine alone was applied.

The effect of 6-azauridine on the synthesis of nucleic acids and proteins demonstrated by semiconductography is in accordance with findings obtained by the classical biochemical methods[10]. Semiconductography thus makes it possible to evaluate rapidly and precisely the distribution of precursors in particular phases of proteosynthesis in early developmental embryonal stages; and furthermore, to examine the quantitative and topical changes of this distribution after application of substances with teratogenic effects. In other words, it is possible to analyse relations between the proteosynthetic activity and morphogenesis[11].

Zusammenfassung. Es wird eine Halbleiterdetektor-Methode beschrieben, durch die ein gleichzeitiger Nachweis der Verteilung von ^{3}H-, ^{14}C- und ^{32}P-markierten Stoffen in Somitten des Hühnerembryons ermöglicht wird. Nachweisvorgang sowie Registrierung werden mittels eines dem System angeschlossenen Computers programmiert.

R. TYKVA, R. JELÍNEK[12] and M. SEIFERTOVÀ

Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Praha 6 (Czechoslovakia),
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[12] Laboratory of Plastic Surgery, Czechoslovak Academy of Sciences, 120 00 Praha 2 (Czechoslovakia).