is well known[1]: The specific dynamic effect of foods can be explained by an acute pituitary stimulating effect.

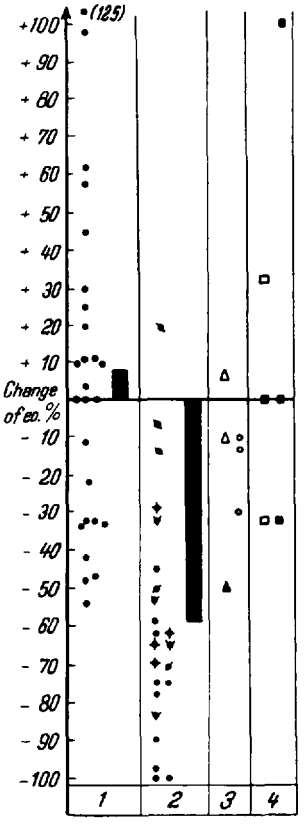

Fig. 1.–Effect of 2 ml human serum (injected s.c.) on the eosinophile-count of fasted rats:

(1) Sera of normal fasted subjects.

(2) Sera taken 4 h after the ingestion of 5 boiled eggwhites (●) or 1–2 g of: Glycin, Valin, Leucin, Tyrosin

(3) Sera taken after the ingestion of 2 g (△) or 100 g (▲) glucose, or 4 g butter (○).

(4) Postprandial sera of patients with Addison's resp. Simmonds' disease (■) compared with their fasted sera (□).

Perhaps the hormones of the blood stream are utilized for the metabolism of the absorbed foods and this is followed by hormone release in the pituitary.

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E. Bencze, C. Sávely, and
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Margit-Hospital, Budapest, May 17, 1954.

Zusammenfassung

Beim Menschen und in Tierversuchen wurde nachgewiesen, dass Eiweiss und verschiedene Aminosäuren Eosinopenie, Blutzuckererhöhung, Ascorbinsäure-Verminderung in den Nebennieren verursachen und bei infantilen weiblichen Ratten gonadotrope Wirkung ausüben. Es wird angenommen, dass die Aminosäuren der Nahrung einen physiologischen Impuls der Hormonsekretion darstellen und dass die spezifisch-dynamische Wirkung der Nährstoffe mit den obigen Befunden erklärlich ist.

[1] P. Constantinides, C. Fortier, and F. R. Skelton, Endocrinology *47*, 351 (1950). – J. Frey and E. Tecklenborg, Klinische Wschr. *30*, 516 (1952).

Detection of Antigens in the Embryo by Labelled Antisera

Coons and Kaplan[1] and Marshall[2] have shown that fluorescent labelled antibodies, applied to fresh frozen or dry frozen tissue, may reveal the localisation of antigens in the tissue. It seemed to us possible that the method might be well suited for the detection of tissue antigens during embryogeny or regeneration. The methods used by the above authors proved suitable when the antigens concerned were well characterised (pneumococcus, hormones, enzymes), while antisera prepared against tissue extracts may be expected to show a wide range of cross-reactions, and it therefore seemed desirable to modify the technique so that it would yield quantitative results. Antisera labelled with radioactive isotopes would seem to be suitable for this purpose. Pressman and Eisen[3] have used antisera labelled with I^{131} or S^{35} for the localisation of tissue antigens *in vivo*; and we have similarly used radioactive antisera, as histochemical reagents, combined with autoradiography, assuming that the density of grains on the film gives an indication of the relative concentrations or specificities of the antigens in different parts of the section.

Organ antisera, labelled by a modification of the method of Pressmann and Eisen with a solution of I_2 in KI^{131}, have been prepared for use on adult and embryonic stages of the house mouse, *Mus musculus*. The same material has also been studied[4] by means of fluorescent labelled antibodies, which might be expected to give better localisation within cells, although not suitable for quantitative estimations. Since some points of embryological interest have emerged, we have thought it appropriate to present a preliminary report; a full account will be published elsewhere.

A number of organ antisera have been made, and so far four of these have been studied; two anti-lens and two anti-cardiac muscle. Serum from a nonimmunized rabbit was used as a control. Both types of antisera showed some cross-reactions *in vitro* with extracts of non-homologous organs; anti-lens, for example, with skin and muscle extracts, anti-cardiac muscle strongly with skeletal muscle and more weakly with skin, liver and other organs. Antisera were applied to the sections both unabsorbed and after absorption with various tissues. The embryos to be investigated were frozen-dried, sectioned, and treated as recommended by Marshall except that, after treatment with the iodinated antisera, the slides were well washed and autoradiographs made as described by Doniach and Pelc[5]. Some sections were afterwards stained progressively with Mayer's haemalum.

Using *in vitro* immunological reactions, Ebert[6] has detected cardiac myosin in the primitive streak stage of the chick before there is any sign of the histological differentiation of the heart. We also find that antigens which react with anti-muscle sera can be detected in young embryos by an increased grain-density over regions of condensed mesenchyme in which histological differentiation has not yet occurred. For example, in a section of a limb of a 15–16 day old mouse embryo (Fig. 1), the areas occupied by the future limb muscula-

[1] A. H. Coons and M. H. Kaplan, J. exp. Med. *91*, 1 (1950).

[2] J. Marshall, J. exp. Med. *94*, 21 (1951); Exp. Cell. Res. *6*, 240 (1954).

[3] D. Pressman and A. N. Eisen, J. Immunol. *64*, 273 (1950).

[4] R. M. Clayton, Nature (in press).

[5] L. Doniach and S. R. Pelc, Brit. J. Radiol. *23*, 184 (1950).

[6] J. D. Ebert, Proc. Nat. Acad. Sci. *39*, 333 (1953).

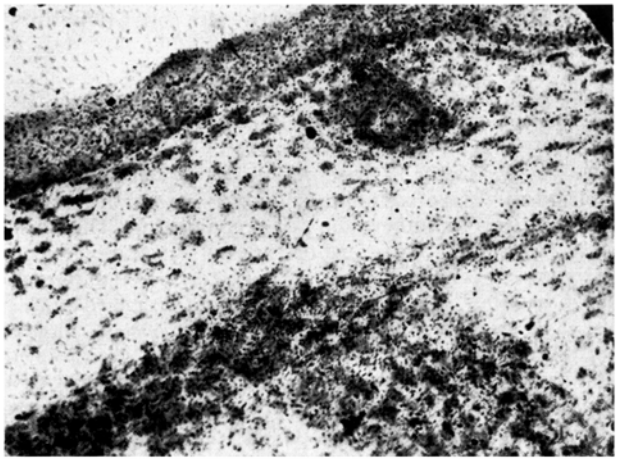

Fig. 1.–Autoradiograph of T.S. of limb of 15–16 day old mouse embryo (× 260). Stained MAYER's haemalum. Focus on grains. The band of condensing mesenchyme shows the highest concentration of grains which can also be seen above individual mesenchymal cells. The guard-hair follicle also shows a high count. A somewhat lower concentration is seen over the epidermis. The intercellular glycoproteins are similar to the background in their grain concentration.

ture and the future panniculus carnosus show a high grain-density; in addition, the periosteum reacts, possibly because of a similarity between periosteum, tendon, and muscle-sheath. The bases of the newly formed guard hair follicles also show a high concentration; it seems likely that this is associated with the condensation of mesenchyme in this region which gives rise to the arrector pili. The intercellular substance, probably a muco-polysaccharide-protein complex, is non-reactive.

The anti-lens antisera, applied to sections of eyes from non-pigmented mice, has consistently shown a very definite distribution of antigens reacting with the antiserum. In the eye from a week-old mouse (Table I), it will be seen that the layers of the retina differ significantly in the amount of antiserum taken up. The strongest activity is in the region comprising the pigmented epithelium and the outer part of the rods (Fig. 2 and 3). The nuclear

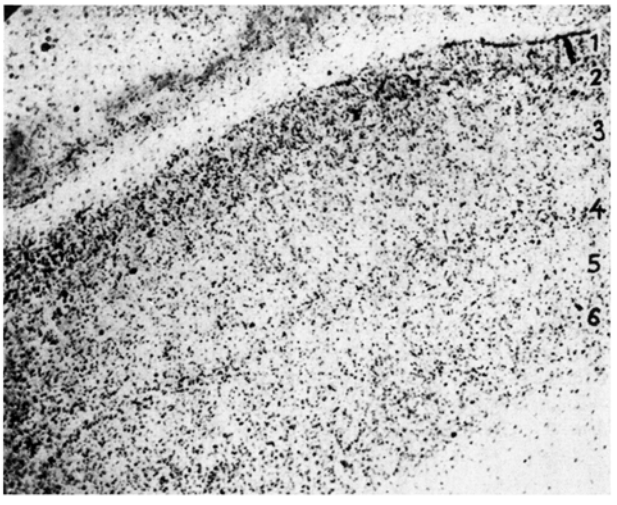

Fig. 2.–Autoradiograph of retina (unstained) (× 360). Zone *1* = epithelium and outer layer of rods; Zone *2* = inner layer of rods; Zone *3* = outer nuclear layer; Zone *4* = outer plexiform layer; Zone *5* = inner nuclear layer; Zone *6* = inner plexiform layer and ganglionic layer and limiting epithelium.

Fig. 3.–Autoradiograph of outer part of retina (× 950). Stained MAYER's haemalum. Zones *1*, *2* and *3* as for Figure 2.

layers of the retina are less active than the fibrillar layers. SATO[1] gives data indicating that the pigmented epithelium can regenerate lens in *Triturus pyrrhogaster*. If this can be shown to be of general significance, the concentration of lens specificity in this region in our preparations may be of interest. Zone 3 (outer nuclear layer) whose nuclei are more close-packed than those of Zone 5, where they are interspersed with fibres, has a slightly lower count than the latter. We cannot yet say whether the cross-reactions of the various layers with lens antisera are in all cases due to the presence of the same or different cross-reacting antigens. In the cornea the anterior epithelium takes up more antiserum than the fibrous cornea (Fig. 4, Table II). The latter is similar in its activity to that of the sclera.

The layers of the choroid and the extrinsic muscles of the eye were found to be much less active than the ectodermal components of the eye. The optic nerve is shown (Fig. 5) to be similar to the fibrillar parts of the retina in its activity. Nevertheless the ciliary muscle, which is ectodermal in origin, has a lower count than that of the extrinsic eye muscles (Table III). The occurrence of low-

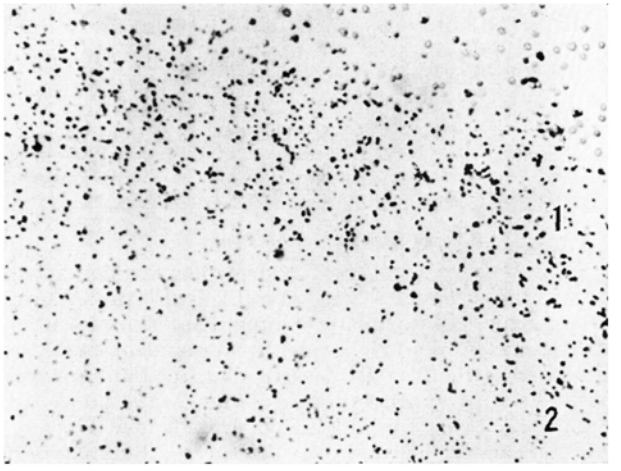

Fig. 4.–Autoradiograph of cornea (unstained) (× 250). *1* = Epithelium of cornea; *2* = Fibrous part of cornea.

[1] T. SATO, Embryologia *1*, 21 (1951).

Table I.–Counts of grains per 64 μ^2 in the retina.

1 Outer layer of rods and pigment epithelium	2 Inner layer of rods	3 Outer nuclear layer	4 Outer plexiform layer	5 Inner nuclear layer	6 Inner plexiform layer to the layer of optic fibres
68·24 ± 2·04	40·36 ± 1·04	20·92 ± 1·01	32·78 ± 1·32	25·48 ± 1·31	38·58 ± 1·35

grade cross-reactions with mesodermal tissues is probably caused by the non-removal of some ciliary muscles during the preparation of the antisera.

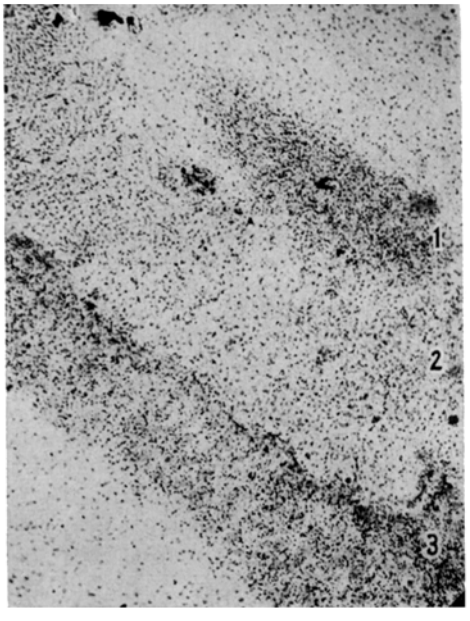

Fig. 5.–Autoradiograph of part of retina and external tissues (unstained) (× 260). *1* = optic nerve; *2* = extrinsic muscles of the eye, chlorid, sclera; *3* = retina. The width of the retina here is about ⅓ of that of the region in Figures 2 and 3.

As might be expected, the activity of the lens was in all cases higher than that of the retinal layers.

Table II.–Counts of grains per 64 μ^2 in the cornea.

Epithelial layer	Fibrous cornea
23·53 ± 1·07	11·63 ± 0·91

Table III.–Counts of grains per 64 μ^2 in the muscles of the eye.

Ciliary muscle	Extrinsic muscle of the eye
26·86 ± 1·06	31·00 ± 1·02

The formation and distribution of these antigens during ontogeny is now being studied. Investigations are also in progress on the changes in quantity of lens specific protein (tested by anti-lens sera after absorption) during induction, development and regeneration.

Acknowledgements. One of us (M.F.) gratefully acknowledges the award of a British Council scholarship. We are indebted to Professor C. H. Waddington for his interest and encouragement, and to several of our colleagues and the technical staff of the Institute of Animal Genetics for assistance with the freeze-drying apparatus, section-cutting, photography, etc.

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Institute of Animal Genetics, Edinburgh, and Department of Zoology, Hebrew University, Jerusalem, July 18, 1954.

Résumé

Des anticorps contre le cristallin et contre le muscle cardiaque de la souris ont été marqués par le radio-iode, puis employés pour la localisation histochimique et pour une analyse quantitative de ces antigènes au cours du développement.

Studies on Factors Influencing the Endogenous Respiration of Liver Homogenates

The Action of Nicotinamide on a Keto-oxidasic Activity of Rat's Liver

We have previously shown how nicotinamide added to a homogenate of rat's liver is capable of increasing endogenous respiration, under particular experimental conditions[1].

As Feigelson and others[2] had previously demonstrated an inhibiting, and not excitatory, action of nicotinamide on endogenous respiration, it was necessary to establish the reason for this divergence in data. We wished to find out if the diet had any influence on these phenomena in order to solve this problem. We have therefore studied the influence of nicotinamide on the endogenous respiration of liver homogenates obtained from (a) rats on a normal diet balanced according to Randoin and Couseret's dictum[3], and the same in a fasting condition, (b) animals fed on a hypoprotein and hyperlipidic diet. The technique used was that already described in previous works[1].

The results of these experiments is that the hypoproteic (hypoprotidic hyperglycidic and hypoprotidic-hyperlipidic) diet and the fasting state (in animals fed on a balanced diet) are the cause of conditions which show up the phenomena already described by us, i.e. endogenous respiration values in the presence of nicotinamide become much higher than those from homogenates without nicotinamide (Fig. 1 and 2). The hyperlipidic diet is responsible for the maximum intensity of the phenomenon (Fig. 1).

[1] L. Villa and N. Dioguardi, Medicina *3*, 287 (1952); Boll. Soc. Lomb. Sci. Med. Biol. *7*, 405 (1952); Com. Congr. internaz. Chim. Biol., Parigi, luglio (1951); Exper. *9*, 469 (1953).

[2] F. Feigelson, J. N. Williams, Jr., and C. A. Elvehjem, J. Biol. Chem. *189*, 361 (1951).

[3] L. Randoni and G. Couseret, Bull. Soc. Sci. d'Hyg. Alim. *1*, 14 (1947).