series. In another series[1], the presumptive ectoderm of early gastrula of *Rana pipiens* was isolated and treated with 0·2 M borate buffer at pH 9·3 for 6–12 min. One hundred and sixty-six isolates thus treated were allowed to reaggregate into 20 larger explants, and cultured at 18°C for 4 to 8 days. Eighteen pieces of such aggregates could be studied in sections, and 23 neural structures were recognized, of which 7 were classified into the brain-type and 2 into the nose-type[2], while others were unorganized neural fragments. All aggregates contained extensive groups of sucker cells as well as epidermis and neural cells. Without close comparative study, the large vacuoles of sucker cells could have been mistaken for notochord vacuoles.

The results of this series do not support the assumption that the secondary axial system in question is caused by a mechanism similar to subcytolytic stimulation of the isolated ectoderm, by showing that the latter causes only archencephalic type or unorganized neural differentiation, and not the spino-caudal type encountered in the secondary axis produced by centrifugation.

The data presented here seem to throw doubt on the assumption that a simple, direct activation of individual cells of the presumptive epidermis by centrifugation is the cause of the formation of a secondary axis. However, the possibilities are not excluded that the centrifugal force affects the presumptive ectoderm directly when it is within the whole intact embryo, perhaps by changing its relationship with other components of the embryo.

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Zusammenfassung

Zentrifugierung der Froschkeime gerade vor oder am ersten Anfang der Gastrulation bringt bekanntlich ein sekundäres axiales System an der ventralen oder lateralen Flanke der Embryonen hervor. Um die Annahme zu prüfen, dass diese spino-kaudalen Gebilde aus dem präsumptiven Ektoderm durch eine direkte Wirkung der Zentrifugierung hervorgehen, wurde isoliertes, präsumptives Ektoderm unter den genau kontrollierten Bedingungen zentrifugiert und gezüchtet. Die Isolate differenzierten Epidermis und Haftdrüsenzellen, aber kein axiales Gewebe. Subzytolytische Behandlung des isolierten, präsumptiven Ektoderms der jungen Gastrulae brachte unorganisierte Neuralgewebe oder archenkephale Gebilde, aber kein spino-kaudales Gewebe hervor. Also gaben die beiden Versuchsreihen keine Unterstützung für die oben genannte Annahme.

[1] The experiment was performed at the Institute for Cancer Research and Lankenau Hospital Research Institute in Philadelphia, by one of the writers, YAMADA, when he was there as a guest investigator. He expresses his sincere thanks to Dr. R. BRIGGS and other members of the Institute for their kind help.

[2] T. YAMADA, Embryologia *1*, 1 (1950b).

On Phosphorylation in Brain Tissue in the Chick

Experiments in the literature relate P_{32} exchange in tissue to the biosynthesis of adenosine triphosphate, a recent report by KREBS[1] presents an approach which differs from those previously employed. In his experiments use is made of sufficiently large quantities of adenosinetriphosphate and inorganic phosphate as a carrier for the label that any fate of the isotope beyond that of its exchange with the adenosine phosphates is negligible in the dilute enzyme system employed. In addition, the organic phosphates are separated by paper chromatography in order that the fate of the label within this system can be well ascertained. However, SLATER and HOLTON[2] believe that the kinetic treatment of KREBS was over simplified. Their criticism was based on an argument involving the system:

$$(1)\quad \text{Alpha-Kg} + \text{CoA} + \text{DPN}^+ \xrightarrow{k_1} \text{Succinyl-CoA} + \text{DPNH} + \text{H}^+.$$

$$(2)\quad \text{Succinyl-CoA} + \text{ADP} + \text{H}_3\text{PO}_4 \underset{k_3}{\overset{k_2}{\rightleftarrows}} \text{Succinate} + \text{CoA} + \text{ATP}.$$

Essentially, if k_2 and k_3 are large with respect to k_1, then exchange of ATP with inorganic phosphate will not measure ATP synthesis. Although the technique might not be sufficiently critical in experiments designed to search for errors in P/O ratios, we believed that such an approach could be suitable to compare tissues with respect to P_{32} exchange. Reported below is an application of the above experimental design in its simplest aspects. P_{32} exchange was examined in liver and brain homogenates from these tissues of the chick embryo.

Three 17 day chick embryos and a day old hatched chick were used for each experiment. The brain and liver were rapidly removed, weighed and placed in beakers containing ice cold saline. These beakers were stored for fifteen minutes at freezing temperatures, homogenized, and sufficient saline was mixed with the homogenate so that for each gm of tissue there would be 20 ml of saline. 4 ml each of liver and brain suspensions were incubated with 12 mg of commercially prepared ATP and 0·2 ml of P_{32} as KH_2PO_4 in a Dubnoff metabolic shaker. At the end of the times indicated below (Table) 1 ml of the enzyme suspension was removed from the beaker, placed into a 15 ml conical centrifuge tube, and the enzyme activity was stopped by adding 0·2 ml of 30% trichloracetic acid. The centrifuge tubes were placed in an ice bath and centrifuged at 5° for 15 min. The supernatant solutions were frozen and stored for the chromatographic separation of the labeled components. When the chromatograms were complete, the paper was cut into one-fourth inch strips after being numbered consecutively, so that the strip having the number one was nearest the origin, and the strip having the highest number was nearest the solvent front. Each strip was then measured for radioactivity using a Geiger tube with an efficiency of approximately 2%. After this measurement each strip was placed in a test tube, 5 ml of water were added, and the optical density at 260 mμ was recorded using the Beckman Model DU spectrophotometer.

[1] H. A. KREBS, A. RUFFO, M. JOHNSON, L. V. EGGLESTON, and R. HEMS, Biochem. J. *54*, 107 (1953).

[2] E. C. SLATER and F. A. HOLTON, Biochem. J. *56*, 28 (1954).

[3] L. V. EGGLESTON and R. HEMS, Biochem. J. *52*, 156 (1952).

Activity of Adenosinetriphosphate Following the Incubation of Liver and Brain Homogenates with $KH_2P_{32}O_4$

Source of Homogenate	Time of Incubation	Optical Density[1] at 260 mμ	Counts per Minute[2]	Cts/min Optical Density Units
	2	0·74	202	273
Liver	5	0·66	260	394
(Embryo)	10	0·75	229	305
	20	0·55	180	337
	2	0·74	122	155
Brain	5	0·72	107	149
(Embryo)	10	0·53	122	230
	20	0·64	92	144
	2	0·50	282	564
Liver	5	0·55	303	551
(Hatched	10	0·50	207	414
Chick)	20	0·23	127	540
	2	0·96	111	116
Brain	5	0·94	108	115
(Hatched	10	0·99	110	111
Chick)	20	0·70	102	146

[1] This measurement represents the absorbance of the leached one-fourth inch strip (see text) from the chromatogram.

[2] Following this measurement of radioactivity on the strip, the solute was leached from the strip in order to measure absorbance.

The data expressed, employing a specific activity function, i.e. counts/min/optical density unit, appear in the table. In all cases the specific activity of the ATP is less in brain than in liver. On the basis of the two groups of experiments shown, the difference between the rate of exchange in brain and in liver appears to be greater in the hatched chick than in the embryo incubated for 17 days. Should subsequent experiments support these findings, it seems that this would be of interest in view of recent differences between the hatched chick[1], and the embryo[2], with respect to carboxylations involving certain acids of the Krebs cycle.

O. M. Gordon, K. O. Donaldson, and L. M. Marshall

College of Medicine, Howard University, Washington, January 15, 1955.

Zusammenfassung

Der Austausch von P^{32} zwischen anorganischem Phosphat und ATP war grösser in der Leber als im Gehirn des Hühnchens. Dieser Unterschied in den zwei Geweben ist jedoch nach dem Ausbrüten des Embryos grösser.

[1] L. H. Newmann, K. O. Donaldson, and L. M. Marshall, Amer. J. Physiol. *178*, 97 (1954).

[2] L. M. Marshall, K. O. Donaldson, L. H. Newman, and M. H. Kahn, J. Biol. Chem. *209*, 697 (1954).

Propriétés chitinolytiques du liquide exuvial du ver à soie (*Bombyx mori* L.)

Lorsqu'on ligature des vers à soie au dernier âge larvaire, entre la tête et le thorax, 48 h après la dernière défécation, on obtient des nymphes non dépouillées de leur exuvie larvaire et qui n'ont pu déglutir leur liquide exuvial (*moulting fluid*). Dans quelques cas, ce liquide s'accumule rapidement dans la région thoracique entre le tégument nymphal et l'exuvie larvaire: en incisant cette exuvie, le liquide sous-jacent jaillit spontanément; il est jaune clair, transparent, mais noircit rapidement au contact de l'air (type I). Plus fréquemment, le liquide exuvial s'accumule lentement dans la région thoracique et noircit au fur et à mesure de son émission (type II). Le prélèvement, réalisé de la même manière que dans le cas précédent, mais plus tardivement, est particulièrement délicat: en effet, le tégument nymphal est fragilisé par la macération dans le liquide exuvial et l'on doit éviter avec soin toute manipulation brusque de la nymphe qui pourrait provoquer des déchirures du tégument et un écoulement de sang.

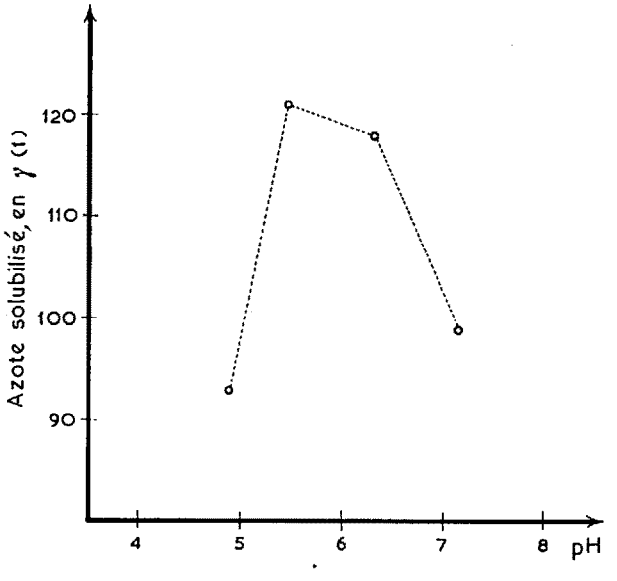

Fig. 1. Activité chitinolytique du liquide exuvial à différents pH. (1): différence entre l'azote-chitine du témoin sans enzyme (139 γ) et l'azote-chitine de chaque test, après 6 h d'incubation.

Conditions expérimentales: 4 ml de liquide exuvial dilué au 1/6; 1 ml de tampon citrate-NaOH 0,6 *M*; 1 ml de suspension de chitine pulvérisée (2,3 mg/ml), à 35°C, en présence de thymol.

Nous avons recherché la présence de chitinase dans le liquide exuvial, par dosage de l'azote-chitine restant après incubation d'une suspension de chitine pure en présence d'une solution de liquide exuvial[1].

Un premier lot de 4 chenilles ligaturées, correspondant au type I décrit ci-dessus, a fourni 4 ml de liquide exuvial. Après dilution au 1/6 par de l'eau bidistillée et centrifugation, on teste l'activité chitinolytique du liquide surnageant. 4 ml de cette solution (soit 0,66 ml de liquide exuvial brut) solubilisent 2,3 mg de chitine, en 14 h d'incubation à 37°C et à pH 6,9. Une station de 2 h à 70°C inactive complètement la chitinase.

Un second lot de 12 chenilles ligaturées, correspondant au type II décrit ci-dessus, permet de recueillir 3,1 ml

[1] Ch. Jeuniaux, Arch. int. Physiol. *59*, 242 (1951).