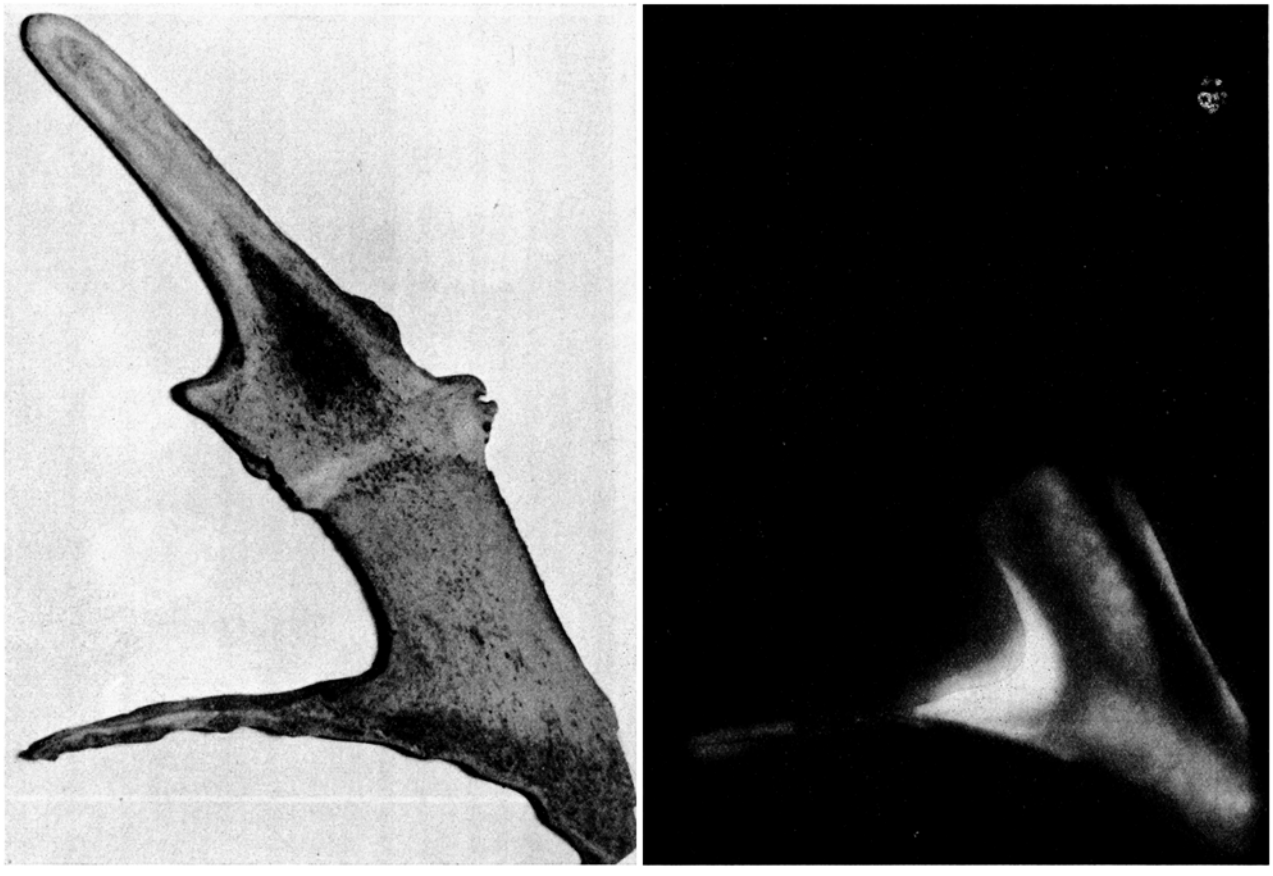

Abb. 2. Schnitt und Radiogramm des konsolidierten Geweihes eines Damhirsches (Tier 2) nach Injektion von signiertem Phosphat.

genügen und die mit der Nahrung zugeführten Mengen Ca^{++} und PO_4^{---} ausreichen. Wir haben bei drei Tieren zu verschiedenen Zeiten den Ca-Gehalt des Blutserums bestimmt. Aus der Tabelle IV ist ersichtlich, dass merkliche Schwankungen nicht vorhanden sind, also auch beim Hirsch während einer starken Aktivierung des Ca- und P-Stoffwechsels das Blut-Ca keine Änderungen erleidet.

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Physiologisch-Chemisches Institut der Universität Basel und Zoologischer Garten, Basel, den 17. Dezember 1952.

Summary

Comparative analysis of functional antlers and bones of the legs in young fallow-deer shows in general the same chemical structure. The amount of N, P, and Ca in the velvet was determined. To confirm the growth in the points of the antlers 2 young stags with growing and functional antlers respectively got injections of radioactive P. The radiograms of the growing antlers cut in thin slices show very clearly an intensive activity at the pointed ends whereas in the functional antler only little activity is seen below the base. The Ca of the blood remains relatively constant.

A Growth Promoting Effect of Cytoplasmic Granules[1]

Treatment of the chorioallantoic membranes of chick embryos with cytoplasmic granules prepared from homogenates of frog or chick embryos has previously been shown[2] to induce cell proliferation and intense basophilic reactions in the membrane cells. These cytological observations were interpreted to be in general agreement with the hypothesis that ribonucleoprotein cytoplasmic particles may induce ribonucleic acid synthesis in the "host" cells and, consequently, the synthesis of proteins. It seemed desirable to reinforce these histological data by attempting to estimate quantitatively the amount of growth induced in the membranes after treatment with suspensions of cytoplasmic granules. The purpose of this report is to present the results obtained from a dry weight analysis of untreated membranes as compared with dry weights of membranes treated with: (1) phosphate buffer/saline mixture; (2) suspensions of "large" cytoplasmic granules (mitochondria); and, (3) the supernatant fluid resulting from the isolation of the granules.

Methods. Preparations of "large" granules from adult frog livers were used in all the experiments. Two grams (wet weight) of tissue were homogenized in 10 cm^3 of phosphate buffer (0·005 M, pH 7·5), the homogenate

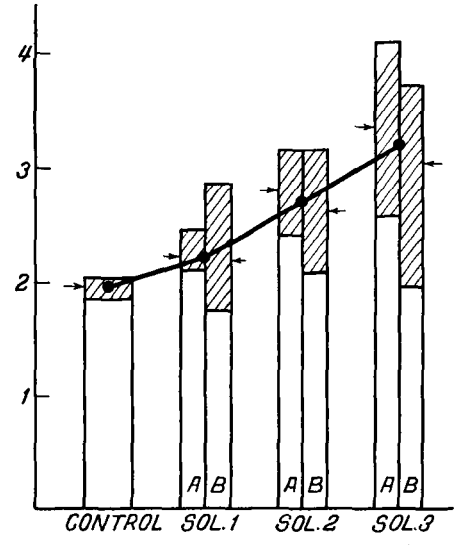
[1] Aided by a grant from the University Research Council, University of Missouri.

[2] J. R. SHAVER and J. BRACHET, Exper. *5*, 235 (1949).

Exp. No.	Untreated		Phosphate buffer/saline				Granules				Supernatant			
	No. cases	Membrane weight mg/cm²	No. cases		Membrane weight mg/cm²		No. cases		Membrane weight mg/cm²		No. cases		Membrane weight mg/cm²	
			0·1	0·2	0·1	0·2	0·1	0·2	0·1	0·2	0·1	0·2	0·1	0·2
1	—	—	9	10	2·10	2·41	8	10	2·89	2·84	—	—	—	—
2	9	2·05	6	5	2·26	2·85	5	6	2·41	2·60	5	4	4·10	3·72
3	12	2·07	4	5	2·45	1·74	5	3	3·14	3·15	6	5	2·57	3·43
4	5	1·78	—	6	—	1·85	—	10	—	2·08	—	10	—	1·95
		1·97			2·27	2·21			2·81	2·62			3·34	3·03

centrifuged at 1500 g twice, and the resulting supernatant fluid centrifuged at 17,500 g for 20 min to isolate the granules, which were then washed twice and suspended in 2 cm³ of a mixture of 1 part phosphate buffer and 9 parts 0·85% NaCl. Preliminary experiments showed that this mixture produced less "non-specific" increase in dry weight than either solution alone. All these operations were carried out at 4°C under sterile conditions. Eggs of as nearly the same size and shape as possible from the same breed of fowl were incubated for 10 days and prepared for the introduction of the solutions by the virological technique of the "false air space". Approximately equal numbers of eggs were treated in one of the following ways: membranes were dropped, but otherwise left untreated; or 0·1 or 0·2 cm³ of solutions 1, 2 or 3, mentioned above, were introduced into their surfaces. Eggs were doubly sealed with "Scotch tape", gently rotated, and incubated for 48 h at 38°C. Membranes were harvested after incubation, washed three times in 0·85% NaCl, and spread on pieces of pure aluminium foil. Foil and membrane were dried to constant weight at 90°C, and a selected area of membrane, free of "non-specific" lesions, large blood vessel junctions, etc., was cut from the large piece. The smaller piece of foil and membrane was weighed, the membrane was washed off with distilled water, and the foil dried to constant weight at 90°C. The weight of 1 cm² of foil having been determined, the weight of membrane per centimeter square was calculated by dividing the weight of tissue by the area of foil upon which it had been spread. The values reported were calculated from the summed weights of the membranes of each class in each experiment. For untreated membranes, it appeared that 10 cases were sufficient to give reproducible results. The response of the membranes to different treatments would be expected to be only approximately alike from one experiment to another, because of variations in preparation of granules, conditions of suspension of solutions on membranes, etc.

Results. The table summarizes the results of four experiments, which are typical of all those performed; the figure, presents these data graphically. In the graph, columns *A* represent data from membranes treated with 0·1 cm³ of solution, columns *B* from those treated with 0·2 cm³; the cross-hatched areas in each column show the extent of the extremes in weight values for each type of treatment; the arrows indicate the arithmetic means in each case, and the heavy line connects the average of the mean values of the two columns. Despite the overlapping of values, especially after treatment with 0·2 cm³ of solution, it is apparent that there is a tendency to increase in weight after each treatment, the increase being greater after treatment with granules than after the phosphate buffer/saline mixture, and greatest after suspension of the supernatant fluid. The average increase of treated membranes, as compared with untreated controls, was 13·7% for solution 1 (phosphate buffer/saline), 37·5% for solution 2 (granules), and 51·4% for solution 3 (supernatant fluid). When these values are corrected for the increase in weight due to the normal growth of the membranes during the incubation period, and when the data for solutions 2 and 3 are further corrected for increase in weight due to the phosphate buffer/saline mixture, it is found that increase due to granules is 18–20%, and that due to supernatant fluid is 30%.



Growth increase in chorioallantoic membranes after treatment. Ordinate: milligrams, dry weight. See text for explanation.

Discussion. These results indicate that "large" granules (mitochondria) from adult tissue (liver) are capable of inducing appreciable growth increase in the embryonic membranes of the chick, and that the supernatant fluid containing the unsedimented microsomes produces a still greater augmentation of tissue. Microscopic observation of sections of treated membranes revealed the same type of cytological reaction as was seen after treatment with embryonic granules[1], previously reported (thickening due to cell proliferation, strong increase in cytoplasmic and nucleolar basophilia, invasion of the mesenchyme between chorion and allantois by the proliferated cells). These histological changes in every case paralleled the dry weight increase, being most pronounced after treatment with supernatant fluid. That the growth increase noted was not due to simple "non-specific" lesions is indicated by the following observations: (1) the cytological reactions of membranes treated with suspensions of fine carbon particles are never as intense as and are of a different nature than those induced by granule sus-

[1] J. R. Shaver and J. Brachet, Exper. *5*, 235 (1949).

pensions or supernatant fluid, consisting mainly in a superficial thickening of chorion cells without accompanying cell proliferation or migration; and, (2) preliminary measurements of dry weight increase due to the carbon particle suspension indicate that it is less than that induced by the phosphate buffer/saline solution (about 5–7% increase over untreated membranes).

Growth promoting factors in cytoplasmic granules have been reported in *in vitro* experiments utilizing microsomes from chick embryo extracts[1], and other evidence also links particulate fractions with increased cellular growth *in vitro*[2]. The intervention *in vivo* of cytoplasmic granules in the promotion of cell division has also been demonstrated in the case of the frog egg, where the injection of cytoplasmic granules into unfertilized eggs will induce parthenogenetic cleavage[3]. What role the granules may play in these processes, however, remains unknown. BRACHET has recently[4] pointed out the possible role of microsomes as agents of protein synthesis in the cell, especially emphasizing their ribonucleic acid content in this connection. It will be noted that, under the conditions of the present experiments, the microsomes would be expected to be in the supernatant fluid, which induced greater growth increase than the granules. What relation these facts have to the ribonucleoprotein content and possible protein synthesizing capacities of these fractions can be found only with further analysis.

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Department of Zoology, University of Missouri, Columbia (Mo.), December 1st, 1952.

Résumé

Des membranes chorioallantoïdiennes d'embryons de poule traitées par 0,1 ou 0,2 cm³ d'une suspension de «gros granules» (mitochondries) provenants d'un homogénat de foie de grenouille, augmentent en poids sec de 20% environ (valeur rapportée à la croissance normale, ainsi qu'à l'effet du tampon phosphate/salin). D'autre part, le liquide surnageant, contenant ses microsomes, produit une augmentation de 30%, dans les mêmes conditions. Dans les deux cas, cette croissance s'accompagne d'une prolifération cellulaire et d'un accroissement de la basophilie cytoplasmique et nucléolaire, effets bien différents de ceux produits par une suspension de fines particules de charbon animal, utilisée comme témoin. Ces résultats sont discutés au point de vue du rôle que jouent les particules cytoplasmiques dans la synthèse des protéines et la croissance cellulaire.

[1] R. TENNENT, A. A. LIEBOW, and K. G. STERN, Proc. Soc. Exptl. Biol. Med. *46*, 18 (1941).

[2] G. BARSKI, J. MAURIN, G. WIELGOSZ, and P. LEPINE, Ann. Pasteur *81*, 9 (1951).

[3] J. R. SHAVER, S. SUBTELNY, and A. WANIA, Biol. Bull. *103*, 282 (1952).

[4] J. BRACHET, Actualités biochim. Liège, Paris No. 16 (1952).

Qualitative Vitamin Requirements for Growth of Larvae of *Calliphora erythrocephala* (Meig)

Introduction. Larvae of *Calliphora erythrocephala* were reared from the egg aseptically, on a diet consisting of casein, Marmite, cholesterol, l-cystine, and water. On this medium the larvae developed into normal imagines. We also obtained good growth if the Marmite in this medium was replaced by the salt mixture according to OSBORNE and MENDEL[1] and a mixture of 9 vitamins based on the composition of Marmite[2], plus vitamin B_{12}.

With the aid of this semi-synthetic diet we determined which of these 10 vitamins are required for normal growth of the larva.

Material and methods. The components of the medium, casein, cholesterol, salts, l-cystine, vitamins, and water were homogenised in an "atomix". The pH was adjusted to 7·0 with some drops of 33% NaOH solution. 40 g portions of this mixture were brought into 200 ml ERLENMEYER flasks with a wide neck. After this the vitamins were added to the flasks, by pipetting them from stock solutions containing all the vitamins or all the vitamins except one. The contents of the flasks were made into a rather solid mass with the aid of cotton-wool. Finally the flasks were plugged with cotton-wool and autoclaved for an hour at 120°C. The following medium was used as a basal diet (Tab. I):

Table I

Vitamin-free casein (Labco or H.L.R.*)			100 mg
l-Cystine** (British Drug House)			4 mg
Salt mixture (Osborne and Mendel)			6 mg
Cholesterol (Comm.)			10 mg
Thiaminchloride (H.L.R.)	3	µg	
Riboflavin (H.L.R.)	6	µg	
Nicotinic acid (H.L.R.)	60	µg	
Ca pantothenate (H.L.R.)	6	µg	
Pyridoxin (H.L.R.)	4	µg	
Folic acid (H.L.R.)	6	µg	
Cholinechloride (H.L.R.)	460	µg	
Biotin (Organon)	0·1	µg	
Inositol (H.L.R.)	180	µg	
Vitamin B_{12} (Organon)	0·015	µg	
Cotton-wool (Comm.)			–
Distilled water up to			1000 mg

* Hoffmann La Roche.

** The omission of l-cystin results in a growth retardation, considerable mortality and abnormal imagines. 2–4 mg l-cystine per gram diet was found to be the minimum quantity for optimal growth stimulation of *Calliphora* larvae. This is in good agreement with the findings of MICHELBACHER *et al.*[3] for larvae of *Lucilia*.

Eggs were obtained by placing fresh meat into a cage, containing the adult flies. About 4–6 h after oviposition the eggs were collected and transferred to a tube containing a solution of 0·9% NaCl and some Rogipon-T[4] in order to decrease the surface tension. With a sterile marten hair brush the clusters of eggs were separated and mixed (since the eggs are laid by different flies, mixing is necessary in order to obtain a homogenous material). The eggs were divided into as many equal groups as there were diets to be investigated in one experiment. After this each group of eggs was poured into a sterile tube and sterilized for 40 min with a mixture of chloramine-T and alcohol according to BLEWETT and FRAENKEL[5]. The sterilizing mixture was removed and the eggs were rinsed with sterile water. After some minutes, the eggs were aseptically transferred into the flasks containing the media to be investigated. In each flask about

[1] T. B. OSBORNE and L. B. MENDEL, J. Biol. Chem. *32*, 309 (1917).

[2] 1 g Marmite from the Marmite Food Extract Company, Ltd., London, contains 30 µg thiamin, 60 µg riboflavin, 600 µg nicotinic acid, 40 µg pyridoxin, 60 µg pantothenic acid, 60 µg folic acid, 4400 µg choline, 1 µg biotin, and 1800 µg inositol.

[3] A. E. MICHELBACHER, W. M. HOPKINS, and W. B. HERMS, J. Exp. Zool. *64*, 109 (1932).

[4] Fa. R. Bosman, Rotterdam.

[5] M. BLEWETT and G. FRAENKEL, Proc. Roy. Soc. B. *132*, 212 (1944).