

Effects of Thyrotropin and Thiouracil on Embryonic Chick Thyroid *in vitro*

It was reported in previous accounts that organ rudiments of the chick embryo develop satisfactorily on a medium consisting solely of adult tissue derivatives¹. In further experiments on these lines a procedure has been evolved by us in which the use of heterologous adult tissue derivatives as the liquid culture medium and a cellulose membrane as the mechanical support for the explants have been introduced². This procedure is but a modification of Fell's classical watch-glass technique for the cultivation of embryonic organ rudiments³.

In the present experiments the embryonic chick thyroid was chosen for cultivation with the liquid medium-cellulose membrane culture procedure.

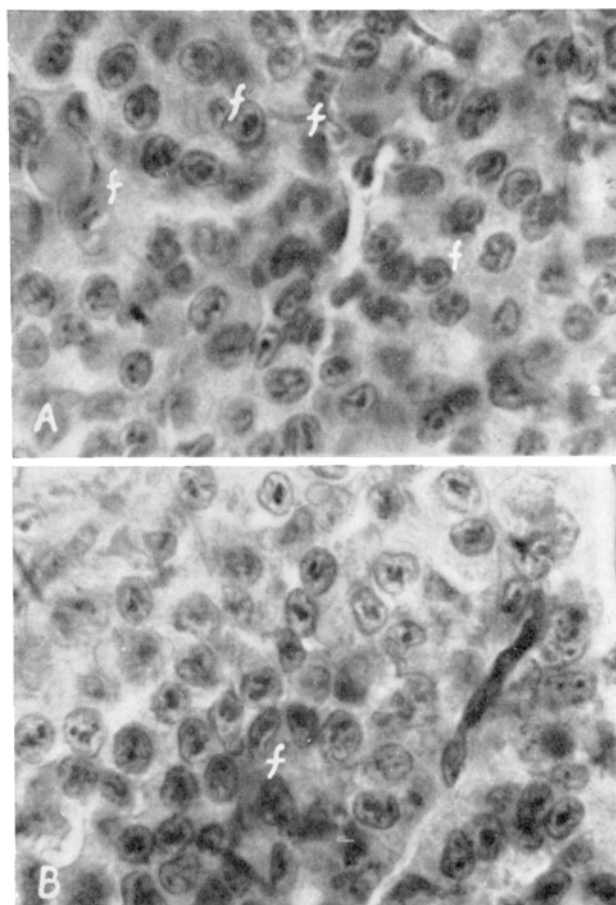
The development *in vitro* of the embryonic chick thyroid was described by CARPENTER⁴. GAILLARD⁵ reported on the effect of thyrotropic hormone on such thyroids in culture, advancing the assumption that the formation of 'colloid' and that of follicles may be dissociated processes. Therefore, explanted thyroid rudiments were subjected to the influence of thyrotropic hormone and thiouracil and their development as regards 'colloid'- and follicle-formation under such conditions was studied.

Results. 20 cultures of thyroids from 8-day chick embryos were grown on the horse serum-ascitic fluid medium. After 2 days *in vitro* droplets of 'colloid' were found scattered throughout the explant. It is difficult to define their location as being intra- or intercellular in position, since histologically the tissue, at this stage, is a symplasmatic structure without cell borders⁶. However, some 'colloid'-droplets appeared surrounded by 3–5 nuclei, the structure suggesting strongly small follicles. After additional 2 days of cultivation follicles were formed, which consisted of 7–9 cells in section, and were filled with intrafollicular 'colloid'. Up to 8 days *in vitro* – after which period the cultures were discontinued – the number of follicles increased steadily.

The series of cultures, in which the effects of thyrotropin (Ambinon, Organon; 0.01 U) and thiouracil (0.4 γ) were studied, was arranged as follows: Both lobes of one embryonic thyroid were cultivated each with a different agent. Thus possible individual differences were excluded and the cultures could be compared as to the effects of the agents. One lobe was grown on a medium consisting of 5 drops of horse serum, 2 of ascitic fluid and 1 drop of Ambinon. The second was cultivated on a similar medium in which 1 drop of thiouracil was substituted for that of Ambinon. In total 36 cultures were grown and examined 18 of each type.

In the cultures containing Ambinon the follicles, formed after 2 days *in vitro*, were considerably larger and comprised more cells than those seen after the same period in explants on horse serum-ascitic fluid medium. They were comparable to those which formed on the latter medium only after 3–4 days in culture, since they consisted of 5–8 cells in section, and were filled with 'colloid'. After 4 days

in vitro there were numerous large follicles in the explant, more than in any other culture examined (Fig. A).



Sections through 8-day thyroid rudiments after 4 days' cultivation: A on a medium containing Ambinon, showing many large follicles filled with 'colloid'; B, on a medium with thiouracil, showing small follicles, devoid of 'colloid'; f = follicle. H.-E., $\times 1140$.

In cultures containing thiouracil after 2 days neither follicles nor 'colloid' droplets were discernable. Only after 4 days *in vitro* very few small follicles appeared in the explants (Fig. B). When compared to the cultures on Ambinon and those grown on horse serum-ascitic fluid only, these explants were greatly retarded with regard to both 'colloid' and follicle formation. Although on the 7th to 8th day of cultivation the differences mentioned seem to become less pronounced, the formation of typical intrafollicular 'colloid' was not evident even at this stage.

Comment. In the thyroid of 8-day chick embryos grown on the horse serum-ascitic fluid medium the first follicles appeared after two days. In comparison with the normal gland the onset of follicle formation was one day earlier. The influence of thyrotropic hormone revealed itself in the early differentiation of follicles, and in their large size at the onset of their formation. The effect of thiouracil was evident in the much delayed differentiation of the follicles and in the almost complete arrest of 'colloid' formation. The differences in development of the embryonic thyroid on various culture media were most conspicuous after the 4th day. The thyrotropin exerted its influence on the onset of the 'colloid' formation rather, than on the follicle development. Thiouracil, on the other hand, retarded both the 'colloid' and follicle formation.

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³ H. B. FELL, J. Roy. micr. Soc. 60, 96 (1940).

⁴ E. CARPENTER, J. exp. Zool. 89, 407 (1942).

⁵ P. J. GAILLARD, Versl. gwone Vergad. Akad. Amst. afd. Nat. 61, 27 (1952).

⁶ W. BRADWAY, Anat. Rec. 42, 157 (1929). – M. L. HOPKINS, J. Morph. 58, 585 (1935).

These findings corroborate GAILLARD's conclusion that the formation of 'colloid' and that of follicles in the embryonic thyroid are dissociated processes⁵. They suggest, moreover, that the thyrotropic and antithyroid agents can exert their respective effects on the embryonic gland *in vitro* already during the stage of histological differentiation. The agents used affected the formation of 'colloid' more markedly than that of follicles.

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Zusammenfassung

Schilddrüsenanlagen von Hühnerembryonen, in Ambion-Medium kultiviert, entwickeln sich schneller und zeigen beschleunigte intrafollikuläre Kolloidbildung. Mit Thiourazil behandelte Kulturen liessen retardierte Follikelbildung und Kolloidbildung erkennen.

The Organic Acid-Soluble Phosphate Contents of Mammalian and Avian Erythrocytes at the Beginning of Postnatal Life

In a previous communication¹ it has been pointed out that the organic acid-soluble phosphate (OAS-P) level in the erythrocytes of the newborn dog and rabbit is very low in comparison to that in the adult animal. This is a finding which is not at all in accord with observations reported by other investigators in various animal species. All the data in the literature available to us indicate that age and the OAS-P contents of the cellular elements correlate just the other way round. The red blood cells of the lamb², the calf³, and the young rat⁴, are richer in components containing OAS-P than the adults of these species. It is on this basis that GUEST and RAPOPORT⁵ regard the relatively higher organic phosphate level of the erythrocytes as characteristic of the young of all mammals. With a view to elucidating the prevailing contradictions our analyses were extended to numerous mammalian species and a number of domestic birds. The present paper incorporates the results obtained.

The organic phosphate fractions were determined from whole blood in the main according to BOMSKOV's method⁶. The corresponding phosphate levels of the red cells were calculated on basis of the hematocrit values. The so-called resistant fraction ($P_{\text{acid soluble}} - P_{180}$) contains the phosphoglycerate-phosphates of the blood. Except for the ruminants, in the red blood cells of the mammals the overwhelmingly greater part of the fraction consists of 2,3-diphosphoglycerate. Simultaneously with the working up of the blood of the newborns, determinations were in every case made from adult animals as well. Since the data we obtained for them agreed largely with the data in the literature, we were content to use a relatively small number of adults. Our findings, presented numerically in the attached Table, appear to permit the following conclusions.

The average values of OAS-P and resistant P in the erythrocytes of newborn and adult animals

Ordo	Species	Group ¹	Number of animals	OAS-P ($P_{\text{ac. sol.}}$ - $P_{\text{inorg.}}$)	Resist. P ($P_{\text{ac. sol.}}$ - P_{180})
				mg/100 ml cells	
Ungulata . . .	Horse	N	4	51.05	39.58
		A	3	51.87	42.17
	Pig	N	9	75.19	35.53
		A	5	102.62	72.30
	Cattle	N	5	42.98	27.30
		A	4	12.05	5.13
	Sheep	N	6	57.77	29.17
		A	3	20.87	3.47
Goat	N	2	58.85	33.25	
	A	2	15.33	6.20	
Carnivora . . .	Dog	N	14	34.71	13.03
		A	5	53.42	40.48
	Cat	N	10	19.16	10.00
		A	3	21.17	14.40
Rodentia . . .	Rabbit	N	20	42.68	16.68
		A	6	80.48	53.23
	Guinea pig	N	6	36.20	26.33
		A	3	49.30	36.20
Primates . . .	Man	N	3	48.30	30.90
		A	3	48.03	33.83
Rasores	Chicken	N	10	90.97	—
		A	3	95.73	—
	Turkey	N	4	119.75	—
		A	3	98.23	—
Lamellirostres .	Duck	N	3	108.43	—
		A	4	116.20	—

¹ N = Newborn, A = Adult

(1) From the point of view raised in the introduction, the mammals we studied can be divided into 3 groups. In 4 species (dog, rabbit, pig, and guinea pig), the red blood cells contain substantially less OAS-P in the newborn than in the adult animal. With the ruminants (cattle, sheep and goat) the situation is just the reverse: in relation to those of the adults, the erythrocytes of the newborns are remarkably rich in OAS-P. The rat too can be classed with this group. Finally, we have man, the dog and the cat, who show no appreciable differences in the two age groups.

The average values for domestic birds agree in the various age groups.

(2) The data in the Table show that from among the organic acid-soluble phosphate compounds, it is the phosphoglycerates (belonging to the resistant fraction), that are responsible for any differences, for the resistant P contents invariably change with the changes in the OAS-P level. Accordingly, in nucleated erythrocytes devoid of phosphoglycerate (avian), the OAS-P level is practically the same in the two age groups.

(3) As early as 1898, considerable differences were described by ABDERHALDEN⁷, in the phosphate contents of the blood of various mammalian species. Since the studies of RAPOPORT and GUEST⁸, the fraction which shows the widest quantitative differences by species, is known to be formed by the phosphoglycerates. Contrary to these conditions prevailing in adult animals, in the red cells of newborn mammals differences according to species of a substantially lower degree were only observed by us. Particularly striking is the great similarity of the resistant

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⁴ S. RAPOPORT and G. M. GUEST, J. biol. Chem. 126, 749 (1938).
⁵ G. M. GUEST and S. RAPOPORT, Phys. Rev. 21, 410 (1941).
⁶ C. BOMSKOV, Hoppe-Seylers Z. 210, 67 (1932).

⁷ E. ABDERHALDEN, Hoppe-Seylers Z. 25, 65 (1898).
⁸ S. RAPOPORT and G. M. GUEST, J. biol. Chem. 138, 269 (1941).