Growth and Mortality Effects Produced in the Early Chick Embryo By Antiserum. (20635)

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The how of ontogeny or epigenesis in the earlier stages of embryonic development is still far from being answered. It is known that at the stage where competency begins organ materials are laid down in a strict and orderly fashion. A series of experiments was designed to investigate the pre-differentiation stages in the chick embryo. A variety of agents was used in an effort to modify the growth pattern of early embryos. The most successful of these agents was found to be whole embryo anti-chick serum produced in hamsters. There are no published reports in the literature which deal with the effect of whole embryo antiserum on the chick embryo. A few workers have reported experiments of this sort on the amphibian embryo(1-3). Previous experiments were done under a variety of conditions and the results are comparable. They may be summarized by saying that the amphibian experiments showed total or partial destruction to the embryos, retardation of growth, production of anomalies and an increased mortality rate in the embryo. The experiments in the present report gave results similar to those in the amphibians. They produced such clearly defined effects that certain new conclusions may be reached. By injection of antigenic embryo materials of specific time intervals, embryonic development in recipient embryos is limited to the stage of the embryo which was used as antigen.

Methods. Fertile eggs, fresh from the yard, were incubated in groups of 100 for 24 hours, 72 hours and 6 days. The eggs were opened with a motor disk and each living embryo removed with a minimum of yolk material and none of the membranes. Technical difficulties are present at the 24-hour stage, the fragility of the embryo at this time necessitates incision of the vitelline membrane and gentle suction removal of the embryo with a sharp, large bore needle and wet tuberculin syringe. The embryo materials after removal were placed in separate lots and thoroughly emulsified in normal saline by syringe suction and expulsion or grinding in a mortar without abrasive. Embryo material of 24 hours, 72 hours, and 6 days was injected intramuscularly into 3 separate groups of hamsters. The injections were started with .5 ml of a rather opaque solution, in which there were particles, in the first injection and increased up to 2.5 ml in the 13th injection. The injections were given every third day and after the last injection a period of 2 weeks was allowed to elapse before the hamster was bled. Those animals which survived the first intracardiac bleeding were rebled on the 21st day after the last injection. The hamster anti-chick embryo serum was tested for sterility and only those lots which were sterile were used in the final experiments. Anti-chick embryo antiserum was not treated with preservative nor were absorption or complement fixation or precipitin tests performed upon the serum. Fresh fertile but unincubated eggs were opened and a window made over the embryo site. In each series approximately .01 ml of anti-chick antiserum was dropped upon the vitelline membrane directly over the embryo site or .01 ml was injected with an 1½ inch, size 26 needle, beneath the membrane into or near the embryo site. There were no differences in the results of these two methods of administration except that a higher mortality resulted in the injected embryos. The same finding of heightened mortality was found amongst the controls where normal hamster serum was injected into the embryo site. We expect this was due to direct damage to the embryo, rather than any antibody effect. Adequate numbers of controls, using normal saline as drop and injection material and normal hamster serum were run. The treated eggs were examined every 24 hours throughout the time of their survival and observations recorded.

Results. 24 hours anti-chick embryo antiserum series. In these eggs embryonic development was markedly retarded and usually

TABLE I. Effect of Hamster Anti-Chick Embryo Serum on the Chick Embryo.

		Developmental stage					
		16	24	72	6 days		
Treatment	No. embryos treated	Blastula gastrula	Prim. streak blood islets	30-35 somites		Abnormalities	Mortality
24 hr	129	25	47	12	2	Cystic growths	43
72 "	168	3	19	41	5	Monsters	100
6 day	60	4	4	5	29	Small retarded embryos	18
Controls	142	0	2	0	0	Normal	7

stopped at the 24-hour stage with death of the embryo at this stage. Occasionally early blood islet formation was found but the embryo rarely progressed beyond this stage. Malformed gastrulae and bizarre-shaped protoplasmic masses appeared. They did not grow well and all attempts to transplant them upon more advanced membranes failed. Rarely an embryo reached the primitive streak stage before death occurred (Table I).

72-hour anti-chick embryo antiserum series: This group developed to more advanced stages than the 24-hour group. There was definite tendency to micro-embryos, some of them reached the heart-beat stage. Malformations of various body parts became more pronounced. Most of these were in the cardiac system with different kinds of vascular and cardiac anomalies, or in the cranial end of the embryo with different kinds of heads and eyes much as micro or macrocephaly, unusually shaped heads and eyes. Occasional 2 or 3 tailed embryos or excessive numbers of limbs were noted. The great majority of embryos were dead by the 72-hour stage: a few survived until the fourth day (Table I).

6-day anti-chick embryo antiserum. Embryos in this group showed some malformations but their number and severity were less than in the previous groups. Some embryos continued to grow until hatching but there was a mortality rate in the group more than twice that of the control series (Table I).

Discussion. Anti-chick embryo antiserum contains a factor which placed in proximity to the early developing embryo produces stoppage of growth in stepwise fashion, anomalies and heightened embryo mortality. The interest here is not in the serological aspect but in the effect upon the embryo's growth pattern. The same kind of experiments done on developing newt embryo explants gave results which caused Clayton(2) to suggest that either an antigen or antigens appear during gastrulation, also the possibility that different parts of the embryo contain common as well as specific antigens. The present report seems to indicate even more clearly that, as the embryo develops new antigens make their appearance. It is certain that the 24- and 72-hour chick embryos contain substances which are antigenic and their antibodies cause development to be reduced and mortality rate to be increased. The mechanism for the above is not clear since the experiments are of limited nature. Critical factors such as concentration of serum, antibody titer and kinds of antibody present, also sites of antibody action must be determined in future experiments.

It may be considered that the appearance of new antigenic material, on a time basis, in the chick embryo is related to the development of new protein complexes. In this field an application of the Schechtman type of studies(4) would be profitable. The present work is suggestive of the development of qualitatively different protein complexes in the embryo concurrent with the embryo's development.

Summary. Using the described experimental conditions whole anti-chick embryo hamster serum placed in proximity to the preincubated chick embryo stops the development of these embryos at a time which corresponds to the time the embryo antigen was obtained. Even though the experimental factors are complex the experimental series differed sharply from the controls. The anti-embryo serum also produced anomalies and a high mortality in the treated series. These experiments show clearly that chick embryos contain distinctive antigenic materials which are related to the embryo's age. The instep order of the results

in the recipient embryos points strongly to the development of qualitatively different protein complexes in the embryo concurrent with the embryo's growth.

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Close-Packed Array of Virus-Like Particles within Cells of a Human Skin Papilloma.* (20636)

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A distinctive type of wart found on the plantar surface and elsewhere on the skin of man has been described previously(1-3). In contrast to the more common wart this papilloma is characterized by certain cytological features including an intranuclear inclusion body and cytoplasmic masses. In addition, virus-like spherical particles measuring 68 μ in diameter were demonstrated in suspensions of ground tissue examined with the electron microscope. This report is concerned with the location and arrangement of these particles in the papilloma cells as seen in thin sections.

Methods. The present report is based upon a study of 3 papillomas. The excised warts were fixed either in osmic acid after the method of Palade(4) or in 10% formalin if the tissue was not available for the former.

Dehydration in alcohol was followed by imbedding in butyl methacrylate with or without added methyl methacrylate (10%) and polymerization by 2-4 dichlorobenzoyl peroxide (2%) by placing in an oven at 60°C for 18 hours. Sections were cut using a glass knife(5) in a Servall cantilever microtome.† They were examined without removal of plastic using an RCA electron microscope model EMU 2E. Sections from the same ribbon were mounted on albumin-coated glass slides, soaked in xylol to remove the plastic, and stained with Weigert's iron hematoxylin or methylene blue to aid in orientation by light microscopy. Staining was carried out by leaving the slide in either dye for one to 2 hours and washing in water. It was then dehydrated in alcohol, cleared in xylol and mounted. Ground suspensions of papillomas were prepared for electron microscopy and shadowed by palladium as previously described(1).

Results. Cells of the different epidermal layers constituting the papilloma are distinguishable in thin sections under the electron microscope since they present about the same features as in conventionally stained paraffin sections and hematoxylin-stained methacrylate sections under the light microscope. The cells in the Malpighian layer of this type of papilloma contain a spheroidal intranuclear inclusion body as well as a few irregular cytoplasmic masses.

Particles resembling the virus-like particles obtained from ground suspensions of these warts have been seen in electron micrographs in the nuclei of cells of the Malpighian layer (Fig. 1-3). These particles are small points at low magnification and spherical at sufficiently high magnification, and generally are arranged in regular rows. The nuclear membrane often appears double and may be separated, apparently by artefact, from the mass of virus-like particles within. It has not been observed to contain virus-like particles. The particles are in straight rows sometimes at right angles to one another, while at others

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† Manufactured by Ivan Sorvall, New York City.