The distance the CAP solution travelled through the yolk sac after replacement of 2 volumes of egg white with 1 volume of CAP solution on the embryo is illustrated in the figure (C). CAP solution enters but does not move beyond the first 5 mm into the yolk sac. The level of radioactivity is 3.5 times lower than that of the group depicted in the figure (B). It may be that as the CAP solution diffuses through the air sac membrane, it stays longer as a cushion below the air space membrane, above the yolk sac, and intermixes with the viscous egg white at a lower rate than an injected solution would. The embryos in all the groups failed to form hemoglobin and to develop. This is an indication that the CAP solution was taken up by the embryos.

Discussion. To draw any valid conclusions in studies dealing with the effect of chemicals on the development of chick embryos in ovo, a substance injected into the yolk sac must be injected next to the sinus terminalis, with the needle directed away from the blastoderm, and to the same depth in the yolk sac in all individual eggs within the same experiment. The advantage of this method is that the desired solution is placed in the yolk sac and in close proximity to the embryo. However, this operation is delicate, because the yolk sac may break. In addition, great care must be taken that the operation is performed in a uniform manner in all individual eggs within the same and related experiments.

Removal of egg white from the egg and addition of the desired solution must be performed gently so that the yolk sac does not change its position in the egg. If the solution is applied not directly above but next to the yolk sac, as it diffuses in a gradient-like manner through the viscous egg white, only part of the solution would reach the yolk sac while most of it would diffuse downwards and outside the yolk sac. It is difficult to remove egg white from the top of the yolk sac without actually pricking it. However, the shortcomings of this method can be overcome by applying the solution on the air sac pit and letting it diffuse passively through the air space membrane. In addition to being easy and repeatable, this passive diffusion method would seem to change the microenvironment of the embryo least since there is no breaking of any of the extraembryonic membranes, as is the case with the other 2 methods.

Effect of different methods of exposure to cyproterone acetate on mortality in chick embryos[1]

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Summary. 33-h chick embryos exposed to 0.25 mg or 0.50 mg cyproterone acetate injected into the yolk sac showed a significantly lower mortality rate than embryos receiving the same dosage applied directly onto the developing blastodisc.

The chick embryo is used extensively as an experimental animal since it is relatively inexpensive and since it allows for manipulation of embryonic tissue not readily accessible in mammalian species. The confined area of the egg chamber containing the embryo and the absence of any maternal influence have enhanced the use of the chick embryo in studies of the toxicity and teratogenicity of numerous chemical agents. However, there is no standard method of introducing chemical agents into the early chick embryo prior to the formation of the chorioallantoic membrane. Methods employed include placing solutions directly on the developing embryo through a 'window'[2-5] or injecting chemical solutions directly into the yolk sac[6,7]. Witschi[8] even suggested placing hormones in crystalline form directly on the embryo. The purpose of this study is to introduce cyproterone acetate, a potent antiandrogen[9], into early chick embryos by 3 different methods and to ascertain whether these methods exhibit similar effects as measured by mortality rates.

Materials and methods. Fertile white Leghorn chick embryos were incubated for 33 h in a forced-draught incubator at 37.5 °C and 60% relative humidity. The embryos were then removed from the incubator and divided into 8 groups. In 5 of the groups a small window was cut in the shell directly above the embryo by means of an electric drill fitted with an emory disc[10]. 2 of these groups received

Toxicity of cyproterone acetate administered by different methods to early chick embryos

Method	Dosage (mg)	Total No. of embryos	No. Living	Mortality (%)
Directly above embryo				
CYP A (Crystalline)	0.25	22	7	68.2
	0.50	16	2	87.5
CYP A (Sesame oil)	0.25	10	2	80.0
	0.50	10	0	100.0
Sesame oil	–	15	3	80.0
Into yolk sac				
CYP A (Sesame oil)	0.25	24	21	12.5
	0.50	20	10	50.0
Sesame oil	–	19	19	0.0

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either 0.25 mg or 0.50 mg crystalline cyproterone acetate (CYP A) placed directly on the blastodisc. Another 2 groups received either 0.25 mg or 0.50 mg CYP A suspended in sesame oil. A control group received only the sesame oil. Preparations in solution were evenly administered onto the entire blastodisc with the aid of a tuberculin syringe.

In the remaining 3 groups a small hole was placed in the blunt end of the egg with an egg punch. A 21 gauge needle attached to a syringe containing the substance to be injected was placed into this opening and passed through the air sac into the yolk sac where the solution was deposited. 1 group received 0.50 mg CYP A suspended in sesame oil and another received half this dosage or 0.25 mg. A control group received only the sesame oil vehicle. Following administration of the test substances, the openings in the eggs were sealed with scotch tape. The eggs were returned to the incubator until the end of the 5th day of incubation (Stages 24–27[11]) at which time the embryos were removed and examined.

Results. The results of this investigation are listed in the accompanying table. Chick embryos exposed to crystalline CYP A had a high incidence of mortality, especially those exposed to 0.50 mg (87.5%). Exposure to CYP A suspended in sesame oil and placed directly above the embryo resulted in 80% and 100% mortality in embryos receiving 0.25 mg and 0.50 mg, respectively. Even sesame oil controls did not escape the high mortality rate. The injection of CYP A into the yolk sac resulted in a significant decrease in the number of embryo deaths when compared to embryos treated directly. Only 3 of the 24 embryos exposed to 0.25 mg CYP A failed to reach 5 days of embryonic life. 50% of those receiving 0.50 mg managed to survive. No deaths were observed in the 19 embryos receiving injections of sesame oil into their yolk sac.

Discussion. Cyproterone acetate exerts its antiandrogenic effects by competitively inhibiting the action of androgens at target sites[12]. The data presented here shows a significant increase in chick embryo mortality when CYP A is placed directly on the embryo in either a crystalline form or suspended in solution when compared to that of embryos exposed to the same concentrations of the chemical injected into the yolk sac. These results differ from those of Caplan[4] who observed that the injection of nicotinamide analogs into the yolk sac of chick embryos resulted in a much higher mortality rate than in those in which the chemicals were placed through a window in the egg directly on the embryo. Another report appeared in which specific sugars placed directly above the chick embryo, exhibited a lesser degree of mortality than did control embryos[13].

These conflicting reports could be due to the fact that these different chemical agents have different absorptive rates in the chick embryo. The fact that even sesame oil, when placed directly over the embryo in the present study, resulted in such a high incidence of mortality suggests that the mere weight of a substance resting on the developing blastodisc may be responsible for restricting the growth of the embryo and resultant death. Applying the test substance directly to the embryo might allow for more rapid absorption than via the yolk sac and might be the method of choice where only short exposure to a drug is required. Studies using different chemical agents are difficult to equate. It is necessary, therefore, that a standard technique of evaluating the toxicity or teratogenicity of chemical agents on the chick embryo be employed.

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Fibrin membrane endowed with biological function. III. Fixing living cells in fibrin gel without impairing their functions

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Summary. Chlorella cells, sea urchin eggs and Paramecium were embedded in fibrin gel which was formed by fibrinogen-fibrin conversion in the presence of thrombin. The embedded Chlorella cells retain the ability of photosynthesis by illumination. The embedded sea urchin eggs develop to normal blastulae and gastrulae. Samples of Paramecium survive for more than several h beating their cilia. It is suggested that this technique of fixing living cells is useful for handling free cells as a mass like a tissue, and for holding free cells in micrurgical experiments.

In previous reports, immobilized asparaginase and immobilized adenosine triphosphatase were prepared by embedding them in fibrin polymer by fibrinogen-fibrin conversion in the presence of thrombin[1,2]. The principal advantages of fibrin polymer as a matrix are as follows; the polymerization of fibrin from fibrinogen proceeds by adding thrombin under physiological conditions (neutral pH and room temperature) and blood coagulation factor XIII serves as a cross-linking reagent between fibrin molecules. In the present paper, the authors succeeded in fixing living cells such as Chlorella, sea urchin eggs and Paramecium in fibrin membrane or block of fibrin gel without impairing their functions.

Materials and methods. Human fibrinogen was obtained from plasma by the method of Blombäck and Blombäck[3], and human thrombin was gifted from the Green Cross Cooperation. Chlorella ellipsoidea cells were grown photoautotrophically at 25 °C by the method of Tamiya et al.[4]. Egg and sperm were obtained from the sea urchin, Hemicentrotus pulcherrimus. Specimens of Paramecium candatum reared in hay infusion at 20 °C were used. Fixing sea urchin eggs in fibrin gel was carried out as

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