indicated that there was minimal associated gastric retention. The gsatric and duodenal mucosa in rats having undergone a parietal cell vagotomy was normal.

Discussion. We have described a simple technique of parietal cell vagotomy in the rat which aimed at denervation of only the acid-secreting part of the stomach. This selective denervation of the body and the rumen in the rat produced an effective denervation of the 'parietal cell mass' and retained the antral and pyloric innervation. Secretomotor function of the stomach, studied after PCV showed a definite reduction in acid secretion, of both basal and secretagogue-stimulation, and the maintenance of gastric 'emptying'. These effects eliminate the complication found after a truncal vagotomy without drainage which results in gross stasis in the stomach[9]. The surgical anatomy of the vagus nerve in the rat allows the operation of PCV to be carried out rapidly with minimal operative trauma, rapid recovery of the rats and without impaired nutrition due to gastric retention.

The technique of parietal cell vagotomy in the rat now provides a method of studying its effect on experimental duodenal ulcers and response to the addition of gastric anti-secretory agents as the H_2-receptor antagonists. Furthermore, the aetiology and pathophysiology of recurrent duodenal ulceration following parietal cell vagotomy can now be studied in the rat.

Application of chemicals in early chick embryos in ovo: A precaution

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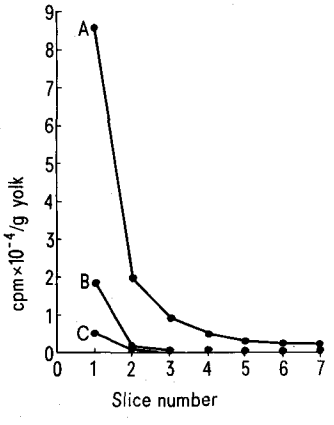
Summary. A passive diffusion method is described and is compared with other methods for the application of chemicals in early chick embryos in ovo.

Work on the effect of chemicals on chick morphogenesis and growth in ovo has mainly used the window method[2], that is, opening a window in the egg and injecting the substance directly under the blastoderm. However, injecting a substance, especially in large volumes, makes the blastoderm lose its intimate and delicate contact with the underlying yolk mass, and results in abnormal development. In the worst cases, it imposes a strong mechanical stress on the blastoderm and makes it blow-up and burst in a baloon-like manner.

To avoid malformations of the developing embryo, the chemical substance must be injected in the yolk sac at a place distant to the embryo. However, the main question is whether the injected material can distribute itself throughout the yolk and reach the embryo in a uniform manner. During the course of a series of experiments, we injected a D-chloramphenicol-^{14}C (CAP) solution into eggs to determine the mode of its diffusion throughout the yolk. In an alternate method, we applied a CAP solution onto the embryo.

Materials and methods. Freshly laid chicken eggs were supplied by a local hatchery. After locating the embryos by candling, a hole was opened at the air space of the eggs. This permitted the embryonic membranes to fall free from the shell membranes. A CAP solution (3×10^{-4} M) which included ^{14}C-CAP (0.04 μCi/ml, 7.94 mCi/mmoles) was either injected into the yolk sac or applied on top of the embryo. For this, either a volume of the solution was applied on the embryo after removal of egg white with a syringe, or the CAP solution was introduced in the pit provided by the exposed air space and was allowed to diffuse through the extraembryonic membranes. The eggs were incubated at 38 °C for 48 h. At the end of the incubation period, they were placed in the freezer until use. The egg white was removed from the frozen egg, and the yolk sac was cut lengthwise into 2 halves. Beginning at the blunt end (slice 1), transparent slices were cut every 5 mm from the right and left yolk halves. The slices were spread on fibre glass filters (Whatman GF/C, 2.5 cm) and radioactivity was determined by liquid scintillation with 10 ml of toluene:flour-Triton-X-100 scintillant. Corresponding slices from the right and left yolk sac halves gave the same counts within 6%.

Results. The figure (A) shows the distance the CAP solution travelled after its injection into the yolk sac. CAP moves very slowly from the place of its injection and is not distributed uniformly throughout the yolk. The figure (B) shows the distance travelled through the yolk sac, after the CAP solution was allowed to diffuse through the air sac membrane. 1 ml of CAP solution diffuses through the air space membrane in 90 min. CAP solution enters but does not move beyond the first 8 mm into the yolk sac.



Distance travelled by 0.6 ml CAP solution (3×10^{-4} M) with 0.04 μCi/ml ^{14}C-CAP (7.94 mCi/mmole) through the yolk sac. *A* The CAP solution was injected into the yolk sac next to the sinus terminalis. *B* The CAP solution was allowed to diffuse through the air sac membrane. *C* The CAP solution was applied onto the embryo after the removal of 1 ml of egg white. Conditions as described in 'materials and methods'.

1 Acknowledgments. We thank Mrs Irene Panagopoulou for expert technical assistance.

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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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