renal arterial pressure is maintained at a constant value by mechanical means. These observations might be explained on the basis of an extrarenal receptor located in the upper aortic distribution, sensitive to diminished blood flow or volume, and regulating renal tubular reabsorption of sodium via humoral or endocrine secretion. Recent experiments have, in fact, disclosed that an increased secretion of aldosterone and hydrocortisone is induced by hemorrhage either directly or via ACTH(2,3). However, the renal retention of sodium elicited by hemorrhage in the present experiments was more rapid than might have been expected as a result of the secretion of presently known adrenal steroids. Furthermore, no changes of potassium excretion characteristic of adrenal steroid action(4) were observed (Table I). Consequently, the present experiments reinforce the previous proposal(1) that hemorrhage may cause an intrarenal redistribution of blood flow, perhaps toward vessels of less than average resistance perfusing tubules with more than average sodium reabsorbing activity. Current studies of the renal hematocrit during isobaric hemorrhage lend further support to this concept.

Summary. The renal excretion of sodium and other renal functions were studied in dogs during non-shocking hemorrhage, while renal arterial pressure was maintained constant by controlled partial mechanical obstruction of the aorta. In some experiments, one kidney was denervated beforehand. The excretion of sodium declined in the absence of measurable changes in glomerular filtration or renal plasma flow. The explanation is proposed that hemorrhage causes an intrarenal redistribution of blood flow which favors tubules with more than average sodium reabsorbing activity, although steroid effects on tubular function cannot be excluded.

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Yolk-sac Perfusion of Chick Embryos: Effects of Various Media on Survival.* (23763)

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The difficulty of maintaining the delicate relationships of the chick embryo to its membranes and to its nutrient supply have until recently precluded direct studies of embryo nutrition in the intact egg. Important data (1) have been obtained indirectly by feeding the laying hen diets deficient in vitamins and minerals, and determining the subsequent effect on development of the embryo. This method cannot be used to study the embryo's supply of amino acids and most energy sources, however, because deficiencies of these nutrients in the hen's diet result in reduced egg production, not in the production of eggs containing lower concentrations of nutrients. One direct approach has been the *in vitro* cultivation technic, which Spratt(2) has used to study the nutrition of chick embryos during early development (18 to 58 hours of incubation). New(3) has recently developed a method of explantation which allows development to proceed for 60 hours, 40 hours of which are *in vitro*. The objectives of the methods developed in this laboratory have been to remove the embryo's nutrients, and

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replace them with a medium of known composition, without disturbing the normal relationship of the embryo to the intact egg. In order to do this, an electrosurgical unit was used to form a channel of coagulated albumen leading through the shell membranes, the albumen, the vitelline membrane and finally into the yolk. The yolk could then be flushed out through the channel and replaced by other yolk or by a replacement medium. When donor yolk was supplied to 3-day chick embryos from which at least 95% of the original yolk had been removed, normal development occurred, including the hatching process(4). After developing the basic technic of yolk replacement we conducted a series of experiments using various media as yolk-replacing materials. Prior to the experiments reported here, however, we had not demonstrated distinct differences among media (except where tolerances for amino acids or heavy metals were exceeded and rapid embryo death ensued). Most of these early trials were performed with defined media similar to that used in the experiments reported in this paper. In this work, the yolk sac was flushed for a few minutes with 15 to 25 ml of medium, after which the hole was closed with plastic adhesive tape. Some embryos withstood 9 flushes of this sort, at 6 hour intervals, before they died. The survival time was the same whether the medium was complete or was deficient in lysine, or whether Earle's balanced salt solution(5) alone was used. We next resorted to continuous yolk-sac perfusion whereby we compared various defined media to balanced salt solutions, but again no differences in survival were observed. Finally, dilute yolk was used in the replacement medium as we report in this paper.

Methods. The basic technics of coagulation and yolk removal have already been described in detail(4). Certain modifications were made to permit the continuous passage of fluids through the yolk sac. In the present study, most of the yolk was removed after 3 days of incubation, but perfusion was not started until the embryos were 4 days old. This schedule permitted the embryo to recover from the operation, facilitated the selection of more uniformly vigorous embryos,

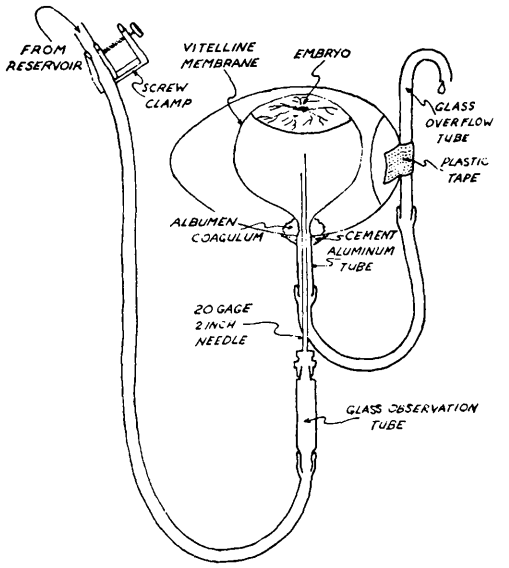

FIG. 1. Yolk-sac perfusion preparation.

and provided time for the residual yolk to accumulate near the opening of the coagulum, with the result that most of it was removed as soon as flushing with the medium was started. After connection between yolk and shell was established(4), an aluminum tube, 4 mm x 15 mm, beveled at both ends, was inserted approximately 4 mm into the hole and was fastened firmly in place with model-airplane cement. Most of the yolk was then flushed out as before, using Earle's balanced salt solution(5). A rubber tube, attached at one end to a glass overflow tube, was slipped over the exposed end of the aluminum tube and taped onto the shell (Fig. 1). These operations were carried out at room temperature, but as soon as the overflow tubes had been attached, the eggs were placed in a room maintained at 100°F and remained there for duration of experiment. The perfusion apparatus consisted of reservoir attached by rubber tubing to stainless steel manifold pipe fitted with small aluminum tubes. Rubber tubing connected these small tubes to glass observation tubes and to 2 in., 20 gauge hypodermic needles. The needles were inserted through rubber overflow tubes and into center of each yolk sac, as shown in Fig. 1. A screw clamp was used to maintain flow rate to each individual egg. *Materials.* Yolk used as part of the perfusion medium was har-

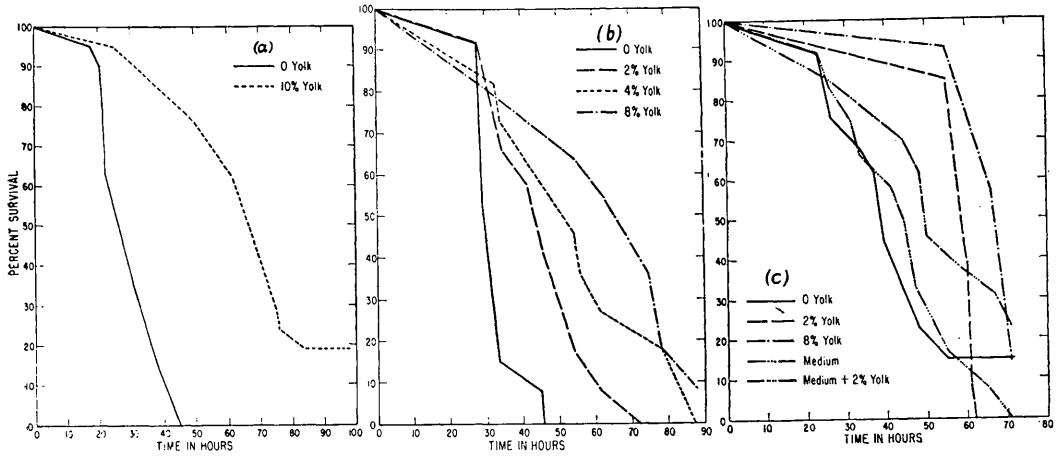

FIG. 2. Effect of varying the perfusion medium on survival of 4-day chick embryos. (a) There were 19 embryos on 0 yolk and 21 on 10% yolk. Perfusion schedule was for alternating 8-hr periods at the rate of 0.5 ml/min. (b) There were 11-13 embryos/group. Same schedule as (a). (c) There were 12-14 embryos/group. Perfusion schedule was for alternating 4-hr periods.

vested aseptically from fresh eggs and was added to Earle's salt solution or to the synthetic medium, which had been sterilized by filtration. The defined medium, which was similar to that of Evans *et al.*(6), contained the following materials, expressed as mg per 100 ml. (The amino acids were all of the L-configuration.): Alanine, 3.38; arginine hydrochloride, 6.30; asparagine, 1.20; aspartic acid, .30; cystine, 2.25; glutamic acid, 3.00; glycine, 8.10; histidine, 3.60; isoleucine, 4.50; leucine, 6.75; lysine hydrochloride, 6.30; methionine, 2.70; phenylalanine, 4.50; proline, 6.00; serine, 3.75; threonine, 3.75; tryptophan, 2.70; tyrosine, 4.50; valine, 4.50; thiamin hydrochloride, .0050; riboflavin, .0050; pyridoxine hydrochloride, .0125; pyridoxal hydrochloride, .0125; niacin, .0125; niacinamide, .0125; calcium pantothenate, .0050; biotin, .0050; folic acid, .0050; choline chloride, .250; i-inositol, .0250; p-aminobenzoic acid, .0250; vitamin A alcohol, .0500; Vit. D-3, .0500; menadione, .0050; d-alpha tocopheryl acetate, .0050; methyl linoleate, .60; cholesterol, .40; Tween 80, 4.50. Earle's salt solution(5) at normal concentration was used in the defined medium as well as in the dilute yolk media. The media and flushing solution contained 66 mg of streptomycin and 9.5 mg of erythromycin per 100 ml. After each 8 hours of perfusion, the media were replaced with fresh mixtures.

Results. The data presented in Fig. 2a indicate that embryos survived for longer periods when perfused with 10% yolk in Earle's salt solution than when Earle's salt solution alone was used. Among the embryos perfused with the medium containing no yolk, death was first observed 18 hours after the start of perfusion, and all had died by 45 hours (141 hours total incubation time). None of the embryos receiving 10% yolk died before 30 hours and some were still alive after 96 hours (192 hours total incubation time), when the experiment was terminated. In this study, the yolk sacs were perfused at a rate of 0.5 ml/min. for 8 hours, then were left undisturbed for an alternate 8-hour period throughout the 96 hours of the experiment. Time of death was estimated by candling appearance, with particular reference to the appearance of the blood vessels.

The second experiment (Fig. 2b) showed the effect of three levels of yolk and of Earle's salt solution alone on survival. The procedures were the same as those of the first experiment. None of the embryos that received no yolk survived beyond 45 hours after the start of the experiment; some of those that received 2% yolk and 4% yolk survived as long as 73 and 87 hours respectively. One of the embryos on the 8% yolk was still alive at 87 hours, when the experiment was terminated.

Fig. 2c shows the results of a study comparing levels of yolk (in Earle's salt solution) and the defined medium. The principal difference from the preceding experiment was that the embryos were perfused for alternating 4-hour periods instead of for 8-hour periods. The embryos receiving no yolk again showed a relatively high death rate, but two of these 13 embryos were alive at the end of the experimental period (71 hours). The defined medium plus 2% yolk allowed poorer survival than the yolk-free defined medium, while the 2% yolk in Earle's salt solution was much better than either of the 2 media containing the known nutrients. The embryos that received 8% yolk survived longer than the embryos on other media.

In all of these studies, the embryos developed normally until death intervened.

Discussion. The results of the second experiment, in which various levels of yolk were compared (Fig. 2b), agree with the data obtained in the first experiment (Fig. 2a). In the third experiment (Fig. 2c) the embryos given 2% yolk or no yolk survived longer than similar embryos in the second experiment. We attribute these apparent discrepancies to incomplete flushing, because at the end of the experiment the yolk sacs of several embryos that had survived on low levels of yolk or on the defined medium contained relatively large amounts of the original yolk.

A comparison of Fig. 2b and 2c, at the 2% and 8% yolk levels, suggests a relationship between the schedule of perfusion periods and yolk concentration. Alternating 8-hour periods (Fig. 2b) may have resulted in more rapid depletion of 2% yolk than of 8% yolk during the 8-hour, non-perfusion period. When perfusion was conducted for alternating 4-hour periods, however, the response may have reflected differences in yolk concentration rather than depletion effects. This would explain the relatively long survival of the 2% yolk embryos in the last experiment.

The results obtained in the present study show that it is technically possible to influence embryo survival by varying the yolk-sac perfusion medium. The defined medium used here, in contrast to very dilute yolk, was clearly not a satisfactory yolk substitute. Its addition to the dilute yolk markedly decreased the value of the dilute yolk medium, thus indicating some imbalance of nutrients, or possible toxicity.

Summary. 1) Yolk-sac perfusion, a new technic which permits the continuous or intermittent passage of fluids through the yolk sac of 4-day chick embryos, has been used to study survival of chick embryos perfused with varying concentrations of yolk. 2) When a yolk-free, balanced salt solution was perfused for 8 hours at 0.5 ml/min., alternating with 8 hours of non-perfusion, almost all embryos died between 20 and 45 hours. Survival time increased in proportion to yolk concentration up to 8% yolk, at which level some embryos were alive 96 hours after perfusion was started. 3) The defined medium was not a satisfactory yolk substitute. The combination of medium and dilute yolk resulted in even poorer survival than that obtained with yolk alone, thus indicating an imbalance or toxicity of one or more components of the medium.

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