

topically labeled precursors should permit the determination of the rate of entry of a given metabolite into the Krebs citric acid cycle in individual organs.

From the therapeutic standpoint, combinations of inhibitors or inhibitors and hormones that act sequentially on metabolic pathways such as protein and nucleic acid synthesis

should be sought. Not only should simultaneous injections (*cf.* Skipper)(21) be attempted, but the individual drugs should also be given separately and in different sequences of administration.

Summary. The action of two or more inhibitors, each of which acts on the same metabolic sequence but upon different enzymes within a limited portion of the sequence, was defined as *sequential blocking*. As a model of such a system, the combined action of malonate and fluoroacetate was studied *in vivo*. It was shown that malonate decreased citrate formation to varying degrees in thymus, kidney, spleen and brain, but increased citrate formation in heart following the injection of fluoroacetate into rats.

§ Note added Jan. 30, 1951: Livers from irradiated male rats were found to accumulate large amounts of citrate following the fluoroacetate treatment(21) in contrast to livers from normal male rats, which do not accumulate citrate. It was also found that normal *female* rats showed a liver response to fluoroacetate and the latter finding has been confirmed in this laboratory, in which preliminary tests with *males* given desiccated thyroid have given liver responses in 4 out of 9 animals, suggesting that several hormone relationships may be involved.

21. Skipper, H. E., *Cancer*, 1949, v1, 475.

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Effect of Yolk Removal in Day-old Chicks on Early Nutritional Requirements.* (18384)

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(Introduced by H. R. Bird.)

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Several investigators have found a positive relationship between early chick growth and the diet of the dam. Rubin and Bird(1) demonstrated that an unidentified growth factor present in cow manure, later shown to be identical with vit. B₁₂, was transmitted through the egg and thence to the chick. Subsequently, Rubin and Bird(2) reported the chick growth factor to be chiefly concentrated in the yolk of the egg. Bethke *et al.*(3), McGinnis and Carver(4), Wiese *et al.*(5), Robblee *et al.*(6), and Csonka and Olsen

(7) also found that the diet of the breeders had a definite influence on the growth of the progeny. It is evident, therefore, that nutritional studies involving the chick are limited by the extent to which the chick can be depleted of the nutrients under consideration. Studies concerning vit. B₁₂ and unidentified chick growth factors conducted by Menge *et al.*(8) in this laboratory have involved the use of chicks from dams fed rations contain-

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1. Rubin, M., and Bird, H. R., *J. Biol. Chem.*, 1946, v163, 393.

2. Rubin, M., and Bird, H. R., *Poultry Sci.*, 1947, v26, 309.

3. Bethke, R. M., Pensack, J. M., and Kennard, D. C., *Poultry Sci.*, 1947, v26, 128.

4. McGinnis, J., and Carver, J. S., *Poultry Sci.*, 1947, v26, 457.

5. Wiese, A. C., Petersen, C. F., and Lampman, C. E., *Poultry Sci.*, 1948, v27, 466.

6. Robblee, A. R., Nichol, C. A., Cravens, W. W., Elvehjem, C. A., and Halpin, J. G., *Poultry Sci.*, 1948, v27, 442.

7. Csonka, F. A., and Olsen, M. W., *J. Nutrition*, 1949, v39, 485.

ing no animal protein supplements and maintained on raised wire floors. Nevertheless, a preliminary period of from 1 to 2 weeks was usually necessary to further deplete the chicks and to standardize the effect of "carry-over" of the nutrient being investigated.

Parker(9), and later Sloan(10) developed a technic for the removal of the yolk from day-old chicks. Sloan termed the operation "deutectomy" and postulated that this procedure could be very well adapted to nutritional studies. A simplified technic for the removal of the yolk from day-old chicks was developed, therefore, to render them more nearly deficient in the nutrients transmitted from the dam. The present paper describes this yolk-removal technic together with the subsequent effect on the early nutritional requirement for vit. B₁₂.

Method of yolk removal. The operative technic as employed for the removal of the yolk from the day-old chick is as follows: The chick is grasped by the legs and placed on its back with the umbilicus toward the operator. It is held in this position with its legs well forward during the entire operation. A solution of 0.5% phenol in 95% alcohol is used to saturate a small area about the umbilicus to sterilize the locus and keep the down in place during the operation. The solution is also used to clean the instruments at regular intervals. A small pair of tweezers is inserted into the umbilicus to enlarge the opening, and an incision of approximately one-eighth inch is made in the anterior portion with small, sharply-pointed scissors. The entire opening, including the umbilicus, should not be over one-fourth inch in length. If the incision is too long, difficulty will be experienced in keeping the intestines within the body cavity after the yolk sac has been removed. On the other hand, if the opening is too small, it may be quite difficult to tease the yolk sac through the incision without tearing. Extreme care must be exercised in making the incision so that only the skin, the

muscular wall, and the peritoneum are cut, while the thin membrane of the yolk sac is left intact. The yolk membrane with its contents is usually visible after this incision is made, for it will tend to force its way through the opening after the peritoneum has been severed. The yolk is easily recognized by its yellow color. Every attempt is made to stimulate the chick to chirp, since this creates an internal pressure which forces the yolk through the opening.

The yolk sac is grasped by the fingers and carefully teased through the opening until the yolk stalk, which joins the yolk sac to the intestine, becomes visible. Instruments have been used with little success in the actual removal of the yolk because the yolk membrane itself is very delicate and is easily ruptured. A certain amount of tearing will be experienced, but in such cases the membrane can be removed with as much of its contents as possible and the remainder of the yolk material forced out of the body cavity by application of gentle pressure to the abdomen of the chick. A small spatula is placed over the cavity and at the juncture of the yolk stalk and intestines. The yolk stalk is then severed with a cauterizing needle. The spatula blade serves to prevent the intestine from being forced through the body cavity and also shields the intestine from the heat of the cauterizing instrument. The incision is then closed with a metal suture. Immediately following the operation, the chicks should be provided a good source of heat to lessen the effect of shock. Most of the chicks exhibit no signs of discomfort within an hour after deutectomy. The fact that the yolk sac is removed through a natural opening contributes to the success of the operation. There appears to be less chance of tearing the yolk membrane and the skin by utilizing the umbilicus in this fashion, for it is here that the muscles have been separated naturally to allow the yolk sac to enter the body cavity of the chick. This type of incision can also be easily closed. After a certain amount of practice, from 2 to 4 minutes were found to be required to deutectomize each chick. The mortality observed throughout was only 4%

8. Menge, H., Combs, G. F., and Shorb, M. S., *Poultry Sci.*, 1949, v38, 775.

9. Parker, Sylvia L., *Hilgardia*, 1929, v4, 1.

10. Sloan, H. J., *Poultry Sci.*, 1936, v15, 23.

in excess of that noted in unoperated chicks.

Experimental. Two experiments were conducted with day-old New Hampshire chicks of mixed sexes to study the effect of deutectomy on their early requirements for vit. B₁₂. The chicks were reared in electrically heated batteries with raised wire floors. Feed and water were supplied *ad libitum*. The diet used had the following percentage composition: cerelose (glucose) 65.67, alpha protein 23, soybean oil 3, salts 1M[†] 6, vit. A and D feeding oil (3000 A, 400 D) 0.5, glycine 1, DL-methionine 0.6, L-leucine 0.2, and protamone 0.03. Each 100 g of diet also contained the following amounts of vitamins (mg): thiamine HCl 1, riboflavin 1, Ca pantothenate 2, niacin 5, pyridoxine HCl 0.6, folic acid 0.15, para-amino benzoic acid 0.2, menadione 0.5, biotin 0.02, alpha-tocopherol 0.5, i-inositol 100, and choline chloride 200. Vit. A (1200 I.U.) and alpha-tocopherol acetate (0.5 mg) were also administered weekly by dropper. This diet, which is quite deficient in vit. B₁₂, was fed to all groups of chicks.

The chicks used in Exp. I were the progeny of hens receiving a ration containing 4% fish meal. Four uniform groups, each containing 20-day-old chicks, were employed. An experimental period of five weeks was used. The chicks in Groups 1 and 3 were deutectomized, whereas the chicks of Groups 2 and 4 were not. The chicks of Groups 1 and 2 received no supplementary vit. B₁₂. Each chick in Groups 3 and 4 received vit. B₁₂ by intramuscular injection as follows: 0.6 γ on the first and eighth day and 1.2 γ on the 15th, 22nd and 29th day of the experiment. The chicks employed in Exp. II were from hens receiving a ration which contained no animal protein concentrates. Seventy-five chicks, 50 of which were deutectomized, were fed the vit. B₁₂-deficient diet for a 2-week experimental period. During this period each chick received 0.6 microgram of vit. B₁₂ by intramuscular injection on the first and eighth day. At the end of this time, one group of unoperated chicks (Group 1) and two groups of deutectomized chicks (Groups 2 and 3)

TABLE I. Effect of Deutectomy on Vit. B₁₂ Requirement of Chicks.

Group No.	Type of chick	Total μ g vit. B ₁₂ in j. per chick during exp. period	Avg gains* during experimental period
Exp. I.			
1	Deutectomized	0	239 (17) [†]
2	Unoperated	0	316 (19)
3	Deutectomized	4.8	322 (18)
4	Unoperated	4.8	321 (18)
Exp. II.			
1	Unoperated	4	261 (12)
2	Deutectomized	4	205 (13)
3	"	8	248 (14)

* Adjusted for sex differences.

[†] Numbers in parentheses refer to number of surviving chicks.

were selected. Each group consisted of 16 chicks, comparable on the basis of general appearance and body weight. During the 3-week experimental period, each chick in Groups 1 and 2 received intramuscular injections of vit. B₁₂ as follows: 1.2 μ g on the first and eighth day and 1.6 μ g on the 15th day. Chicks in Group 3 received twice the amount of vit. B₁₂ at each injection.

Results and discussion. The results of Exp. I and II are presented in Table I. In Exp. I the deutectomized chicks which received the vit. B₁₂-deficient diet alone (Group 1) grew less rapidly than did the unoperated chicks fed the same diet (Group 2). However, when vit. B₁₂ was administered to deutectomized and unoperated chicks fed the same diet (Groups 3 and 4), no difference in average gain was obtained. It is evident, therefore, that removing the yolk from day-old chicks by means of deutectomy resulted in the removal of vit. B₁₂ or another substance possessing vit. B₁₂ activity. The results of Exp. II confirm the observations noted above since the deutectomized chicks required twice as much supplemental vit. B₁₂ as did unoperated chicks in order to support a similar rate of growth. These observations are in agreement with Yacowitz *et al.* (11) and Mil-

[†] Described by Briggs, G. M., *J. Nutrition*, 1946, v31, 79.

11. Yacowitz, H., Peeler, H. T., Miller, R. F., Carlson, C. W., Heuser, G. F., and Norris, L. C., Informal Poultry Nutrition Conference, Atlantic City, N. J., April, 1950.

ligan and Combs(12) who showed that vit. B₁₂ activity is transmitted from the dam through the yolk of the egg. These workers also demonstrated that the diet of the dam had a direct influence on the amount of vit. B₁₂ activity contained in the egg yolk.

The growth response of deutectomized chicks as compared with that of unoperated chicks in both experiments demonstrates that the yolk-removal operation had little or no effect on growth when the diet of the chick was adequately supplemented. Although no evidence was obtained in these trials to demonstrate the removal by deutectomy of nutrients other than vit. B₁₂, this procedure is considered to be of value as a means of reducing the initial "carry-over" of nutrients from the dam in day-old chicks.

Summary. An operative technic for the removal of the yolk from day-old chicks has been described. This procedure was developed to render chicks relatively free of the nutrients transmitted through the yolk from the

dam. Observations on the growth rate of deutectomized chicks as compared with that of unoperated controls show that the operation had little or no effect on growth when an adequately supplemented diet was supplied. The results of these trials demonstrate that vit. B₁₂ or a substance possessing vit. B₁₂ activity was present in the yolk of the day-old chick and that it was removed by deutectomy.

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12. Milligan, J. L., and Combs, G. F., *Poultry Sci.*, 1950, v29, 772.

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Critical Evidence that Vagal Stimulation Does Not Release Gastrin. (18385)

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At least 2 mechanisms have been shown unequivocally to result in the gastric secretion of hydrochloric acid: (a) A vagally mediated mechanism, which is physiologically activated by the thought, sight, smell and ingestion of food; by stimulation of the vagus nucleus chemically with insulin-induced hypoglycemia; or by electrical stimulation of the vagal trunks directly; and (b) a chemical mechanism, which is physiologically activated by the presence of food in the antrum of the stomach or by distention of the antrum and which is followed by the release of the hormone, gastrin. These mechanisms have generally been considered to be independent of each other(1) although, in the normal course

of ingestion and digestion of food, they may operate synchronously as well as successively. In recent years, however, the view has been advanced that a more direct relation exists between the nervous and the hormonal phases of gastric acid secretion. Uvnäs(2) has presented evidence from which he concluded that vagal stimulation during the neural phase of gastric secretion leads to the release of the antral hormone, gastrin. This hypothesis is extremely attractive, since it unifies the

1. Dragstedt, L. R., Woodward, E. R., Storer, E. H., Oberhelman, H. A., Jr., and Smith, C. A., *Proc. Soc. Exp. Biol. and Med.*, 1950, v73, 676.

2. Uvnäs, B., *Acta Physiol. Scand.*, 1942, v4, Supplement 13.