TABLE III. Effect of Thiocarbanilide on Egg Shell Color-Score and Plasma and Yolk Concentrations of DNC during Feeding of Nicarbazin.

% nicarbazin in feed	% thiocarbanilide in feed	Shell color score period Cont.	Exp.	DNC conc. Plasma, γ/ml	Yolk, γ/g
.008	None	2.9	.5	2.45	9.34
	.002	2.5	1.7	.99	11.10
	.004	3.2	2.8	1.16	5.70
	.008	2.7	2.8	.96	5.07
.0120	None	3.2	.5		20.55
	.003	2.8	.2	1.98	21.20
	.006	3.7	1.9	1.54	15.50
	.0120	2.1	2.1	.78	9.52
	.020	2.9	2.8	.33	1.25
.0125	None	3.5	.5	2.02	20.4
	.0125	3.5	3.5		
	.025	2.9	2.3	.52	1.85

creased plasma and yolk DNC levels reflected a decreased assimilation of nicarbazin by the laying hen because of the thiocarbanilide in the feed.

Discussion. The anticoccidial and egg shell depigmenting effects of nicarbazin are related to the amount of DNC absorbed from the intestinal tract. Plasma levels of DNC are correlated with both of these effects, which in turn are proportional to concentration of nicarbazin in feed(4,6). Thiocarbanilide effectively antagonized the anticoccidial activity and shell depigmenting effect of the structurally related component (DNC) of nicarbazin. The antagonism occurred either by feeding or injecting thiocarbanilide and simultaneously feeding nicarbazin. Thus, the decreased plasma and yolk DNC concentrations caused by thiocarbanilide appear to be due to an interference with nicarbazin absorption.

Summary. 1) Thiocarbanilide, in a 1 to 1 ratio or less, effectively inhibited the anticoccidial activity of nicarbazin when given parenterally as well as orally. 2) Thiocarbanilide also antagonized the effect of nicarbazin on the depigmentation of egg shell color. 3) In both instances, the antagonistic effect of thiocarbanilide was correlated with decreased plasma concentrations of dinitrocarbanilide. 4) It is suggested that thiocarbanilide may interfere with the activity of nicarbazin through competition in absorption.

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Influence of Beta-Aminopropionitrile upon Development of Connective Tissue in Croton Oil Pouches. (23048)

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Injection of air and dilute croton oil into subcutaneous tissues of rats produces a well-defined pouch(1). The wall of the pouch after 6 days is composed principally of fibroblasts and minimal numbers of blood vessels. When beta-aminopropionitrile (BAPN) is fed to rats daily in drinking water, the development of a croton oil pouch is retarded. Because BAPN retards development of croton oil pouches, it seemed desirable to examine gross and microscopic findings in these pouches, and to analyze some important constituents of connective tissue to resolve whether there are also differences in concentration of such components. The 2 components selected for analyses were hexosamine and hydroxyproline. These constituents were chosen because hexosamines are an im-

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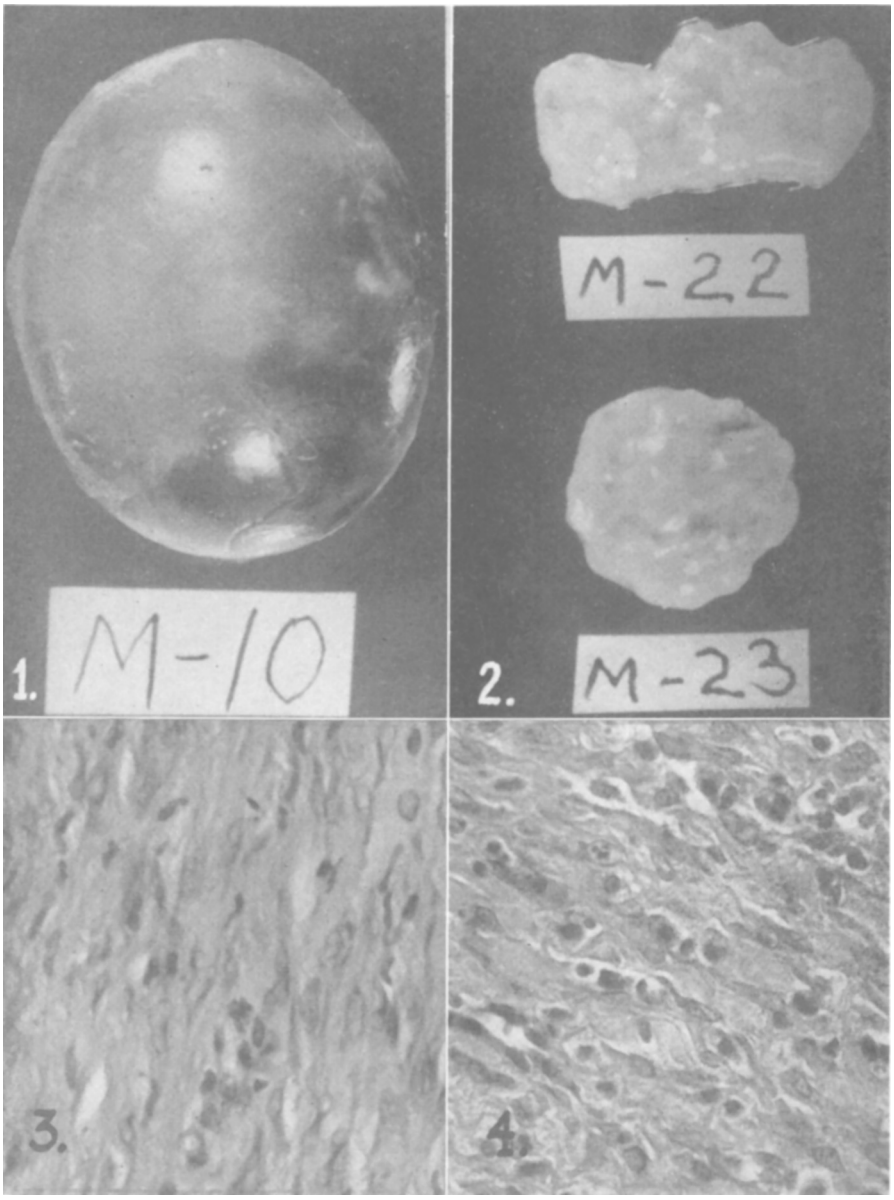

FIG. 1. Eighteen day croton oil pouch from control rat approximately actual size. Note relatively smooth exterior and well developed capsule.

FIG. 2. Two 18 day pouches from test animals are collapsed, smaller and their surfaces irregular.

FIG. 3. Section of capsule from control pouch stained with hematoxylin and eosin. Fibroblasts tend to align along one plane. Many nuclei are fusiform and collagen is abundant. × 350.

FIG. 4. Immature fibroblasts with round nuclei are numerous and collagen synthesis is diminished. × 350.

portant component of ground substance(2); whereas, hydroxyproline is indicative of concentration of collagen(3).

Method. Rats were fed a commercial diet.† Each day, to every 100 ml of drinking water, 0.2 ml of concentrated alkaline BAPN kept

† Purina Laboratory Chow, Ralston Purina Co., St. Louis, Mo.

at 4°C was added, and this was offered to 18 rats with croton oil pouches. Three control and 3 test rats were killed after 6, 12, and 18 days. The pouches were carefully dissected from surrounding areolar tissue, care taken to exclude visible blood vessels. The inner surface of each pouch was scraped to remove fibrin and inflammatory cells. After several washings in cold (2-4°C) physiologic saline, the pouches were weighed and sections made for microscopy. Thereafter, the pouches were cut into 2-4 mm pieces and placed into 5 ml of cold 4% perchloric acid. At this stage, pouch walls with areas of hemorrhage or necrosis were discarded. The tissues were frozen in liquid air and pulverized in a mortar. The pulverized protein was then subjected to extraction in perchloric acid, ethanol, and ether as recommended by Muller and Herranen(4). Total volume of perchloric acid washings was recorded. Filtered aliquots of perchloric acid washings were subjected to micro Kjeldahl digestion, and total acid soluble nitrogen calculated. Lipids were extracted from the protein by repeated ethanol and ether extractions. Total dry, fat free protein residues were weighed in each case. Ten mg of protein was hydrolyzed in 1 ml of 4 N HCl acid for 15 hours at 100°C. These acid hydrolysates were used for determination of hexosamine by the Boas method(5) and hydroxyproline concentration by the method of Neuman and Logan(3). Aliquots of acid hydrolysate were also subjected to micro Kjeldahl digestion to determine per cent of N in the protein residue.

Results. The results are shown in Tables I and II.

Gross. The test pouches were poorly formed, ill-defined from surrounding areolar connective tissue, and many were collapsed (Fig. 1). They were smaller, both in size and in wet and dry weight as compared to corresponding control pouches. Comparable quantities of oil were obtained in collapsed and control pouches. The pouch from only 1 of 9 rats (No. 16) was comparable in weight to those of control rats (Table II).

Microscopic. The microscopic findings are summarized in Table I. The presence of an

TABLE I. Microscopic Observation of Croton Oil Pouches from Normal and Test Rats Fed BAPN.

Rat No.	Fibroblastic maturation	Schiff positive material	Organization
	Control, Day 6		
1	2+	2+	2+
2	2+	3+	+
3	3+	2+	+
	Day 12		
4	4+	+	2-+
5	3+	+	2+
6	3+	2+	3+
	Day 18		
8	4+	+	4+
9	4+	+	4+
12	4+	+	4+
	Test, Day 6		
13	+	4+	+
14	+	3+	+
15	+	3+	+
	Day 12		
16	2+	3+	+
17	2+	3+	2+
18 & 24*			
	Day 18		
25†	+	4+	2+
29†	2+	4+	+
33†	+	4+	+

* Tissue used for chemical study only.
† From a second series of rats which were given 0.15 instead of 0.2 ml/100 ml of drinking water.

increase in number of fibroblasts with large spherical nuclei associated with less collagen fibers in the stroma per unit area is interpreted as a delay in the rate of maturation of fibrocytes. Such alterations were more pronounced in test pouches. An attempt was also made to evaluate the degree of fibroblastic maturation (1-4+) at 6, 12 and 18 days depending upon the abundance of collagen and the presence of immature (spherical) or mature (fusiform) nuclei. The organization or alignment of fibroblasts and collagen fibers parallel to the surface of the pouch wall was also investigated. Interference with alignment of collagen fibers, and fibroblasts with respect to the surface was more pronounced in the test pouches (Fig. 2 and 3). An approximation of fibroblastic orientation in relation to time was graded from 1-4+ and tabulated. In addition to alterations in the quantity of collagen and cellular variations larger foci of homogeneous eosinophilic material were present in test pouches. Dupli-

TABLE II. Hexosamine and Hydroxyproline Concentrations in Croton Oil Pouches from Normal and Test Rats Fed BAPN.

Rat No.	Wet wt of pouch, mg	Wt of extracted protein, mg	N. in extracted protein, %	Acid extractable N., % total N.	Hexosamine in extracted protein, %	Hydroxyproline in extracted protein, %
			Control, Day 6			
1	1810	138.6	14.2	8.36	1.02	2.70
2	1120	68.9	13.7	9.28	.96	2.96
3	1130	106.2	13.7	10.00	.93	2.97
			Day 12			
4	1720	174.8	14.3		1.03	3.98
5	1700	159.4	14.7	4.1	.88	3.80
6	1740	149.6	13.9	3.4	.94	3.53
			Day 18			
8	1410	124.2	12.9	4.95	.92	4.26
9	1320	119.9	13.2	4.52	.98	4.78
12	1340	97.1	13.7	6.82	.98	4.16
			Test, Day 6			
13	540	44.6	14.6	13.6	.90	1.89
14	650	33.9	13.3	19.5	.93	1.65
15	1000	57.4	13.1	20.1	1.00	1.65
			Day 12			
16	1450	122.5	14.3	11.6	.92	3.66
17	670	37.8	14.4	18.8	1.06	2.48
18 & 24*	810	62.5	14.0	14.9	1.17	2.94
			Day 18			
25†	580	42.5	13.5		1.18	1.88
29†	530	26.8	14.1		.82	3.52
33†	810	35.9	14.1		.98	3.52

* Pouches from 2 rats were combined.

† From a second series of rats which were given 0.15 instead of 0.2 ml/100 ml of drinking water.

cate sections were stained with periodic-acid Schiff in each case(6). Examination disclosed that Schiff positive material which is localized in the homogeneous foci is more abundant in the test pouches.

Chemical. Weights of pouches before and following extraction as well as hydroxyproline and hexosamine concentrations are shown in Table II. Cold perchloric acid extraction will dissolve salts, nucleotides and amino acids. Acid extraction might also dissolve some collagen leaving insoluble collagen for analysis(7). More acid soluble nitrogenous material was obtained from the test than from control pouches. Whether this difference is due to extraction of nucleic acids from the immature fibroblasts or the presence of greater quantities of an acid soluble protein matrix in the stroma of the pouch wall is not known. Nitrogen in the fat free protein residue varied from 12.9 to 14.4%. It is not possible to make any correlation between variations in % N and age of pouch. The hexosamine concentration varied from 0.92 to 1.02 in control pouches. A variation of this degree is probably inherent in the method of hexosamine determination. Although amount of hexosamine in test pouches varied somewhat more widely, the values appear comparable to those observed in controls.

The hydroxyproline concentration is expressed as per cent of protein residue because we wished to investigate variations in hydroxyproline with respect to protein concentration. As one would anticipate, the hydroxyproline concentrations are lowest in 6-day pouches and gradually increase in concentration with time in the control group. In addition to reductions in wet weight and extracted protein residue, there is also less hydroxyproline in the dry, fat free protein from

the test pouches. Examination of hydroxyproline concentration shows that the levels are less in test pouches at 6, 12, and 18 days. Whether hydroxyproline is expressed in per cent of protein residue or in terms of per cent protein N, the concentrations are lower in test pouches. Less hydroxyproline is evident in 8 of 9 test rats. This decrease in concentration of hydroxyproline in test pouches is in agreement with our microscopic observations of delayed collagen synthesis.

Discussion. Feeding BAPN in drinking water results in smaller pouches when compared to those observed in control rats. In addition to gross and microscopic observations, it is also possible to demonstrate less hydroxyproline in the extracted protein from test pouches. Abundant quantities of homogeneous eosinophilic material between immature fibroblasts might either be ground substance or a collagen precursor. The fact that hexosamine concentrations were not significantly increased in the extracted test pouches suggests that the eosinophilic material either is not ground substance or that the mucopolysaccharides were dissolved during perchloric acid extraction. The hexosamine concentrations of extracted protein residue from the pouch are approximately double the values reported by Boas for areolar tissue in young rats(5). Increased hexosamine concentrations in croton oil pouch may be related to the presence of immature and metabolically active fibroblasts.

Microscopic observation of abundant homogeneous eosinophilic material with reduced numbers of collagen fibrils, recoveries of proportionately less fat free protein and lower concentrations of hydroxyproline in the extracted protein from test pouches, all suggest that BAPN in some way exerts an influence on collagen synthesis. The observation of increased solubility in perchloric acid of pulverized protein from test pouches also suggests that they differ from normal. Since the acid extractable nitrogenous material was not analyzed for hydroxyproline it is not possible to say whether there is more acid soluble collagen in the test pouches.

The fibroplasia which is stimulated by croton oil is sufficient to permit chemical analysis with the added advantage that the approximate age of fibroblasts is known. Uncorrected collagen of the skin represents 67.8% of dry fat free weight when calculated from the hydroxyproline content(3). In our studies the hydroxyproline concentrations varied from 1.65 to 4.78% of extracted protein. These concentrations when expressed in collagen equivalents indicate that collagen varied from 14.1 to 35.6% of extracted protein. Robertson and Schwartz have reported delayed collagen and hydroxyproline synthesis in scorbutic guinea pigs injected with extracts of Irish moss(8). In their experiments the delayed synthesis of collagen is related to deficiency of vit. C. BAPN probably effects collagen synthesis in some other way because the rats in our study did not develop any signs of vit. C deficiency.

Summary. Pouches from control and test rats fed BAPN were removed after 6, 12, and 18 days. Extraneous material such as collections of leukocytes, fibrin, and areas of hemorrhage were removed before the pouches were extracted in 4% perchloric acid, ethanol, and ether. Grossly the test pouches were poorly formed, and microscopically they showed decrease in fibroblastic maturation and collagenic fiber formation with relative increase in homogeneous eosinophilic Schiff positive material. Fat free protein residue was analyzed for hexosamine and hydroxyproline concentration. More nitrogenous material was consistently obtained from test pouches during perchloric acid extraction. Hexosamine concentration in different pouches varied from 0.82 to 1.17% of dry fat free protein. Significant alterations with age or variations from control values were not observed. In control pouches the hydroxyproline concentration gradually increased to approximately 4% in 18 days. Hydroxyproline concentrations in the test pouches were significantly less than in controls of corresponding age.

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A Simple, Inexpensive, Continuously-Variable Infusion Pump.* (23049)

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The instrument to be described was developed to fill the need for an infusion pump with which rate of delivery could be continuously varied over a wide range during injection. The present pump has met this need and has been employed for 9 months in experiments on small animals in which rate of injection has been critical.

Details of construction and mode of operation of this pump will be apparent from Fig. 1 and 2. The motor (Bodine Electric Co., Chicago, 1/50 H.P., 1725 rpm, with gear reduction to 11.5 rpm) turns the first drive cone (A). The 4 identical drive cones are knurled cast aluminum. The conical portion is 4" long and the large and small diameters are 3" and .95" respectively. Cone A drives cone B by means of rubber ring (¼" by 18" circumference) which provides contact between the 2 cones. The point of contact of the ring with the cones is adjustable by means of yoke K, rod L and setscrew M (Fig. 1). It can be seen that when the largest portion of cone A is driving B, B rotates 3.16 times as rapidly as A; conversely, when the smallest portion of A is driving B, A rotates at 3.16 times the speed of B. Thus, a 10-fold range of speed is achieved by the first pair of cones. Cone C is coupled to B and both turn at the same speed. Cone D, however, is driven by C through a mechanism identical to that linking cones A and B. Range of speed for the 2 pairs of drive cones is therefore 100-fold. To prevent slippage of the cones on the rubber rings it is necessary to have considerable tension on the 2 pairs of drive cones. This is achieved by means of the tension frame (N to P) which is clearly shown in Fig. 2. This frame carries cones B and C on supports N and N′ and holds them under tension against cones A and D by means of the 2 springs on rod O. The springs are turned in opposite directions, and tension on them can be varied by means of nut and setscrew P.

Cone D turns the threaded ½" shaft F through the sprocket and chain drive E (Fig. 1). The syringe plunger is driven by rider G which is carried along as shaft F turns. This rider is made of two brass bars ½" x 1" x 4" which are clamped together and drilled and threaded to receive shaft F. The 2 halves of the rider are then hinged at one end and closed with a pivoted pin at the other end so that they can be opened and moved on F. Also on this rider is bar I which can be set to contact switch J and stop the pump at any desired position. The syringe is held in place by the lucite block H. Two cylindrical lucite plugs attached to the holder project through holes in the pump mounting plate. The holder is removed by lifting vertically. It is convenient to make several holders with identical mounting plugs to accommodate several syringe sizes. About 10 seconds are required to remove the syringe, refill

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