may be an explanation for the satisfactory cell propagation in starch, glycogen, maltose and sucrose, as suggested by Harris(8). The ability of the 2 dialyzed equine sera and the inability of the 3 human sera to hydrolyze sucrose sufficiently to support cell growth tends to support this view.

The difference in the sucrase activities of the various individual sera is of interest in studies of the nutritional requirement of human cells. It illustrates how the results of certain studies may be influenced by the source of serum incorporated into the assay medium. Other differences of sera have already been described(9).

Summary. 1) Under the described experimental conditions, both conjunctival and HeLa cells propagated satisfactorily in the following individual carbohydrates: starch, glycogen, maltose, d-glucose, d-fructose, d-mannose and d(+)galactose. Cell propagation in sucrose depended largely on source of dialyzed serum. Sodium pyruvate, sodium lactate, d-ribose and d(+)xylose were unable to support cell multiplication, though cell degeneration in the presence of these carbohydrates and intermediates was definitely retarded. L(+)rhamnose, l(−)fucose and l-sorbose appeared to delay the degeneration of the HeLa cells more effectively than that of the conjunctival cells. Lactose, l(−)xylose, d(−)arabinose, l(+)arabinose, d-2-deoxyribose, i-erythritol and dihydroxyacetone neither supported growth nor retarded degeneration. 2) Glucose at low concentration of 0.1 mM is capable of supporting cell growth for at least several months. Net increase in number of cells at a concentration of 0.1 mM is significantly lower than that in 10 mM glucose. When pyruvate and alanine were added to the medium, the concentration of glucose could be further reduced to 0.01 mM without resulting in cell degeneration. D-mannose and d(+)galactose both at 0.01 mM concentrations, and d-fructose at somewhat higher concentration, are effective substitutes for glucose.

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Effect of Beta-Aminopropionitrile on Incorporation of Glycine by Croton Oil Pouches in Rats.* (23472)

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Although several investigators have studied the influence of beta-aminopropionitrile (BAPN) on mesodermal tissues, the mechanism of the BAPN effect is still not understood. Dasler(1) pointed out that it is tissues which are high in collagen that appear to be primarily affected by the lathyrus factor, and he has suggested that lathyrism involves a disturbance in the metabolism of connective tissue or of a connective tissue component. Ponseti and Shepard(2) also concluded that mesenchymal tissues are influenced by the lathyrus

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factor, the active principle of which is BAPN (3).

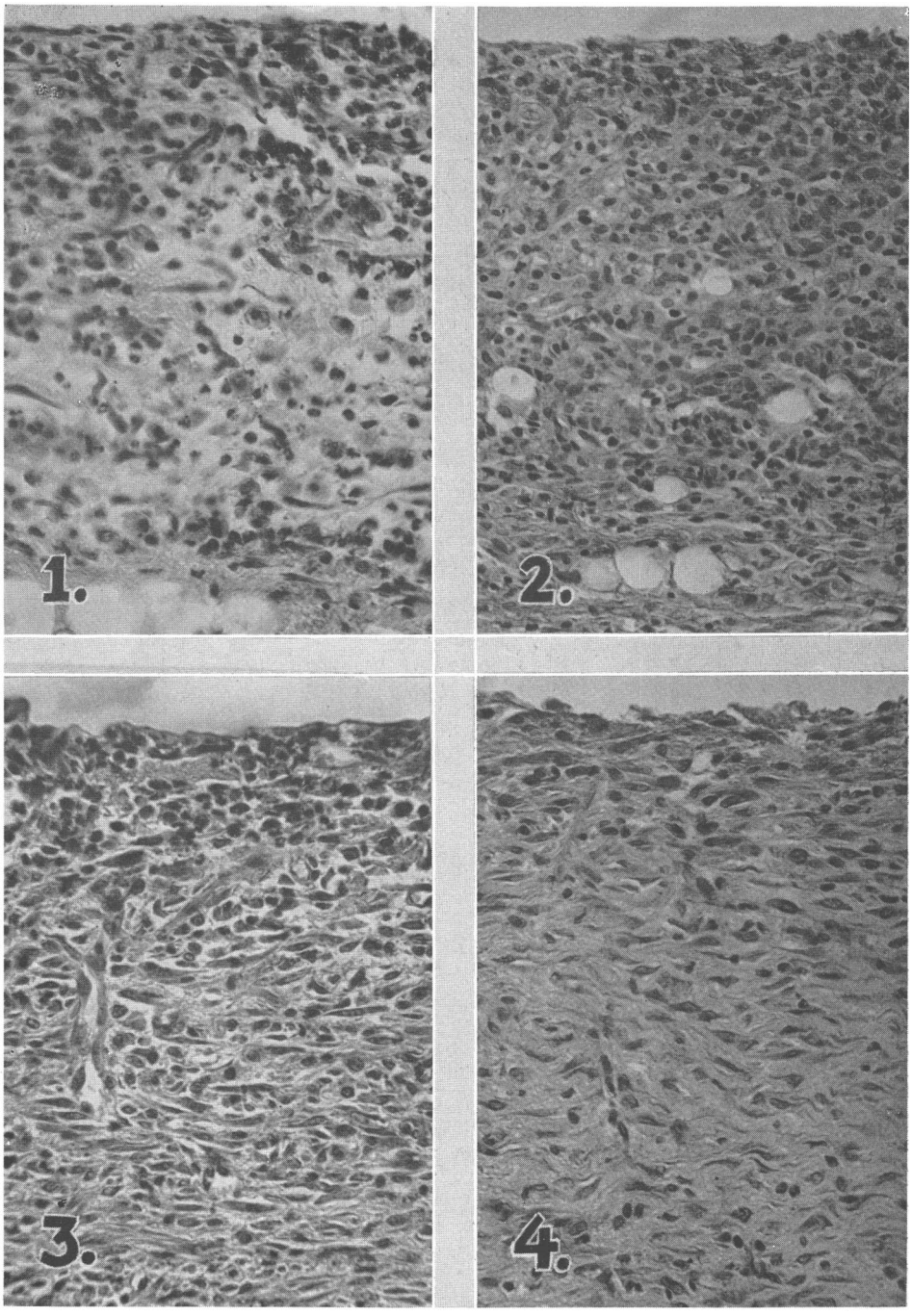
A previous study carried out in this laboratory has shown that the use of Selye's croton oil pouch technic provides an excellent tool for demonstrating the influence of BAPN on newly formed connective tissue(4). This technic has been used in the present investigation to determine the effect of BAPN on incorporation of radioactive glycine into croton oil pouches and dermis of rats.

Methods. Croton oil pouches were produced in female Sprague-Dawley rats according to a modification of Selye's procedure(5). Rats weighing 80 to 90 g were injected subcutaneously in the dorsal region with 20 ml of air followed by 1 ml of 1% croton oil. *In vivo* studies using radioactive glycine were carried out on 12 rats with croton oil pouches. Six test rats were fed 40 mg of mono beta-aminopropionitrile fumarate† (BAPN) in 20 ml of drinking water daily for 9 days, while control rats received no BAPN. A commercially prepared rat pellet diet‡ was fed during the course of these studies. All animals were weighed at the beginning of the experiment and again before sacrificing them on the ninth day. On the eighth day of pouch development each rat received 2 intraperitoneal injections of 50 μc of carboxyl-labeled glycine§ 24 and 15 hours before the time of sacrifice. On the ninth day the animals were decapitated, and the croton oil pouches were cut open and placed immediately into 0.9% NaCl to remove croton oil and hemorrhagic inflammatory exudate. After wet weights were obtained, the tissue was cut into 4 to 5 mm pieces and placed into cold 0.9% NaCl until time of pulverization. Three x 6 cm sections of skin adjacent to the pouch were also taken. The segments were laid upon a flat surface, excess fat was removed, and the dermis was scraped off with a scalpel. The dermis was weighed, cut into pieces, and placed into cold NaCl solution. Tissues were frozen in liquid air and pulverized by means of a stainless steel mortar and pestle. The powdered tissue was then suspended in 10 ml of cold 4% perchloric acid to extract free amino acids, nucleotides, and other acid soluble substances. The suspension was centrifuged and the supernate saved for radioactivity measurements. The protein residues were resuspended twice in 10 ml portions of cold 4% perchloric acid, centrifuged, and the supernate added to the acid soluble fraction. In order to be certain that the residues were free of acid soluble substances, they were rinsed twice more with cold perchloric acid and the washings discarded. Lipids were extracted from the residues with 10 ml portions of 80% ethanol, 95% ethanol, two 10 ml portions of absolute ethanol, and two 10 ml portions of ether. The lipid-containing ethanol and ether extracts were pooled and radioactivity measurements made on these fractions. The ether extracted protein residues were allowed to dry in a desiccator overnight. A measured amount of the dried residue (3 to 5 mg) was then plated on tared aluminum planchets using 2 or 3 drops of water to distribute the residue evenly. The samples were counted in a Geiger-Mueller windowless gas flow counter. Radioactivity was expressed as counts per minute per milligram of protein. A correction for self-absorption and counting efficiency was made. Extraction and hydrolysis of nucleic acids was accomplished by treating the protein residues with 4% perchloric acid at 90°C for 30 minutes. Purine bases present in the perchloric acid extract were eluted from a Dowex 50 column with 6 N HCl according to the method of LePage(6). Guanine and adenine were separated by descending paper chromatography using a solvent system of tertiary butyl alcohol and constant boiling HCl as described by Markham and Smith(7). After locating the bases on the chromatographic strips with the aid of ultraviolet light, guanine and adenine were eluted with 1 N HCl. The eluate, which was collected in small beakers, was then evaporated to dryness with a stream of air, plated in .01 N HCl, and the radioactivity counted. In order to calculate the concentration of purine on the planchets, optical densities in 0.1 N HCl were

† Mono Beta-aminopropionitrile Fumarate was obtained from Abbott Laboratories, North Chicago, Ill.

‡ Purina Lab Chow.

§ Glycine-1-C^{14} was purchased from Tracerlab, Inc., allocated by U.S.A.E.C.



Formalin fixed sections of croton oil pouches removed from rats after 3, 6, 9 and 15 days. Before fixation fibrin and inflammatory cells were removed from the inner aspect of the capsule, which is shown at top of each photomicrograph. Sections were stained with hematoxylin and eosin. × 350.

FIG. 1. Three-day-old croton oil pouch. There is a mixture of fibroblasts and leukocytes in the capsule. Stroma is poorly developed.

FIG. 2. In the 6-day-old capsule immature fibroblasts with round or oval nuclei predominate. Stroma is more dense than at 3 days. Several cystic spaces can be seen in the capsule.

TABLE I. Radioactive Glycine Uptake into Proteins of Rat Croton Oil Pouches and Dermis.

	Wt at start of exp., g	Net gain, g	Wet wt of pouch, mg	Protein residue of pouch (—cpm/mg protein—)	Protein residue of dermis (—cpm/mg protein—)
Exp. 1					
Controls	84.5	36.0	1220	5760	1905
	91.8	28.5	1310	5130	1610
	97.6	27.7	560	3430	1685
Test	91.4	23.5	520	3900	1559
	92.5	25.5	660	3790	1392
	89.1	22.4	600	3390	1416
Exp. 2					
Controls	86.0	13.5	790	4920	2280
	85.1	20.4	720	4420	1760
	79.6	16.9	670	3060	1500
Test	84.0	18.5	500	3590	1744
	80.2	15.8	400	2750	2100
	83.5	17.5	350	2580	918

determined in a Beckman DU spectrophotometer at 260 and 250 millimicrons for adenine and guanine, respectively. The concentrations were then calculated according to the molar extinction coefficients used by Markham and Smith(8). Radioactivity was expressed as counts per minute per micromole of adenine or guanine.

Five mg aliquots of the protein residues from croton oil pouches were hydrolyzed for 15 hours at 100°C with 4 N HCl. Following hydrolysis the samples were evaporated to dryness at 40°C under a stream of air and redissolved in 0.5 ml of water. Two 25λ portions were applied in 4 cm adjacent bands on Whatman No. 1 filter paper buffered with a mixture of 1 M HAc and 0.6 M HCOOH at pH 2.3. After 2 hours' electrophoretic migration at 14000 volts the filter paper strip was cut lengthwise to separate the duplicate samples, and amino acids were located by dipping one of the strips in 0.2% ninhydrin in acetone. The amino acids of the duplicate strip then were eluted from segments which corresponded to the ninhydrin bands of the developed strip. The eluates were plated and the radioactivity counted.

Results. Microscopic studies of the croton oil pouch. A preliminary time study was carried out to establish the phase of pouch development at which there are maximum numbers of young fibroblasts. Croton oil pouches were removed from 2 animals at 3, 6, 9, 12, and 15 days. Pieces of each pouch were fixed in 10% formalin, embedded, sectioned, and stained with hematoxylin-eosin. Microscopic findings demonstrated that the capsules of 3-day pouches contain many inflammatory cells (Fig. 1). By the sixth day almost all inflammatory cells have disappeared. The capsule is principally composed of immature fibroblasts containing large, round vesiculated nuclei. Minimal numbers of collagen fibers are present at 6 days (Fig. 2). At 9 days the capsule contains more collagen. The fibroblasts now contain round and fusiform nuclei (Fig. 3). On the twelfth day of development fusiform fibroblasts and small oval nuclei are conspicuous. The ratio of collagen to cellular elements is markedly increased. By the fifteenth day the capsule is composed mostly of collagen. Mature fibroblasts with fusiform nuclei now constitute over 75% of all fibroblasts (Fig. 4). Feeding BAPN to

FIG. 3. At 9 days fibroblasts with fusiform nuclei are conspicuous for the first time. Immature fibroblasts with round nuclei are more numerous near internal border of capsule. Many fibroblasts oriented parallel to the lumen. A capillary is evident in this segment of the pouch wall. Stroma is still poorly developed although collagen fibers are apparent.

FIG. 4. Fifteen-day-old capsule has a well defined collagen stroma. Most of fibroblasts are of adult type with fusiform nuclei. Few immature fibroblasts with round nuclei can still be seen at inner border.

rats delays maturation of the fibroblasts and delays collagen synthesis. Since maximum numbers of young fibroblasts are present at 7 to 9 days, the 8-day-old pouch was chosen as a source of connective tissue to study metabolic behavior of radioactive glycine as influenced by BAPN.

In vivo studies using radioactive glycine. The results of duplicate experiments 1 and 2 indicate that BAPN exerts a significant effect on rate of incorporation of radioactive glycine into proteins of granuloma and dermal tissues of rats. The data in Table I demonstrate from 23 to 28% less uptake of glycine in capsules of croton oil pouches from BAPN treated animals. Somewhat less affected by BAPN is rate of glycine incorporation into the dermal protein: those from BAPN fed animals have approximately 15% less activity than controls. The protein residues from both croton oil pouch and dermis show high specific activities. As one might expect, the uptake of radioactive glycine into the proteins of rapidly developing connective tissue, such as the croton oil pouch, is almost 3 times as great as in the more slowly metabolizing dermis.

Electrophoresis of the protein hydrolysates separates the following amino acids: aspartic acid, glutamic acid, methionine, threonine, serine, alanine, and glycine. Only in the serine and glycine bands is there significant radioactivity. The results show that, even though there is less total radioactivity in the protein residues from BAPN fed rats as compared to controls, the percentage of the radioactivity accounted for as glycine and serine in both test and control pouches is the same. Fifty-three % of the radioactivity is found in the glycine band in both the control and treated croton oil pouches. Thirty-six % of the radioactivity is accounted for by serine.

The incorporation of radioactive glycine into the purine bases of nucleic acids is given in Table II. The data of duplicate experiments demonstrate that the specific activities of guanine and adenine are essentially the same in both the control and BAPN treated tissues. On the basis of these experiments it appears, then, that BAPN does not affect the rate of incorporation of radioactive glycine into the purine bases of nucleic acids in croton oil pouches and dermis of the rat, whereas significant differences in the incorporation into protein are evident. The fact that animals continue to grow despite administration of BAPN suggests that the effect is a relatively local one, showing selective specificity in producing extracellular protein.

TABLE II. Radioactive Glycine Uptake into Nucleic Acids of Rat Croton Oil Pouches and Dermis.

	Adenine of pouch	Adenine of dermis	Guanine of pouch	Guanine of dermis
	cpm/μM adenine		cpm/μM guanine	
Exp. 1				
Controls	1753	1470	2750	2100
	1390	1525	2040	2290
	1230	1585	1800	2340
Test	1305	1493	1900	2250
	1420	1583	2160	2120
	1300	1680	1830	2030
Exp. 2				
Controls	1756	1583	2990	3030
	2000	1454	2680	2560
	1430	1650	1985	2660
Test	1550	1895	2120	3230
	1353	2700	1530	4150
	1370	1475	2010	2150

A preliminary analysis of the acid soluble fraction indicates only a small uptake of glycine into acid soluble substances. Measurements of radioactivity in the lipid fraction indicate that almost no labeled material is incorporated into this fraction.

Discussion. Our experimental data are compatible with the findings of Mielke, *et al.* (4) who demonstrated that collagen synthesis is in some way inhibited by BAPN. Their experiments indicated differences in the chemical composition and weights of croton oil pouches from animals fed BAPN as compared with controls. Test pouches contained from 20 to 25% less hydroxyproline in protein residues prepared similarly to those in the present investigation, the hydroxyproline determinations indicating amounts of collagen present. These workers also found that pouches from BAPN treated animals weighed only half as much as control pouches; the present work indicated similar results.

Utilizing glycine-1-C^{14} we have shown that BAPN significantly decreases amino acid incorporation into the protein residues of croton

oil pouches and dermis, but incorporation into the purines of nucleic acids from either of these tissues is unaffected. The data here reported do show some variation in response, which might possibly be accounted for by individual animal variations. It is interesting to note, however, that in croton oil pouch protein residues, the activity of the tissue is directly proportional to the weight of the pouch. Where the weight is high, as in the control tissues, specific activity is also high. One control tissue which, for some unknown reason, did not respond to the croton oil injection, showed lower radioactive glycine incorporation, similar to the incorporation found in test pouches.

To evaluate its specific role in connective tissue metabolism, we have attempted to determine whether BAPN acts primarily on regulation of cellular uptake or utilization of amino acids in collagen synthesis. Since glycine uptake into acid insoluble purines is unaltered by feeding BAPN, it would appear that BAPN does not exert its effect at the level of nucleic acid synthesis in the cellular component of the connective tissue. On the other hand, the observed lowering of glycine incorporation into the protein residue is compatible with earlier findings indicating an inhibitory action of BAPN on the synthesis of extracellular collagen by these same cells.

Summary. 1. An investigation of the influence of BAPN on incorporation of glycine-1-C^{14} into proteins and nucleic acids of rat croton oil pouches and dermis was undertaken. 2. Incorporation of glycine into protein residues of fibrous tissue from croton oil pouches was decreased about 25% in animals pretreated with BAPN. In protein residues of dermis the rate of incorporation of glycine was also decreased, but to a much lesser degree, about 15%. 3. Studies of the nucleic acid fraction indicate that no change occurs in glycine uptake in fibrous tissue of dermis or croton oil pouches from BAPN treated animals as compared to controls. 4. The results of these experiments suggest that BAPN primarily affects amino acid incorporation into collagen or ground substance rather than the cellular portion of connective tissue.

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Thresholds for Pain and Convulsions in the Guinea Pig Following Massive Whole-body Irradiation. (23473)

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The guinea pig shows an abrupt shortening of survival time and a striking change in mode of death(1) when a single whole-body radiation dose exceeds 6,000 r (6 Kr). Below 6 Kr the clinical picture is essentially that of depression, while above 6 Kr excitation, with frequent convulsions, is regularly seen. Pentobarbital sodium, given prior to radiation, prevents the clinical signs of excitation(2) and lengthens survival time. The observable neuropathology(3) appears insufficient to account for the early death and this suggested the present study on the functional state of some accessible neurological systems.

Previous studies(4) have shown only small and subtle effects on the electroencephalogram at doses below 6 Kr and paroxysmal seizure waves at higher doses. Changes in the elec-