react with the same labeled antiserum, either prior to or following the vaccination procedure. These results are being reported elsewhere(10).

Summary. Tissue culture cells infected with various enteroviruses show specific immunofluorescence when stained directly with conjugated fractionated antiserum. The indirect method of fluorescent antibody determination is an even more satisfactory diagnostic technic since it makes possible the detection and titration of enterovirus antibodies actively acquired following clinical or subclinical infection or from vaccination.

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Relationship Between Soluble Collagen and Urinary Hydroxyproline in the Lathyritic Rat.* (27665)

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Ziff and coworkers(1) have demonstrated increased excretion of urinary hydroxyproline peptides in children as compared with adults. Decreased excretion has recently been observed in this laboratory in non-growing children (2). This was corrected in a pituitary dwarf by administration of human growth hormone. Increased levels of urinary hydroxyproline were observed in a patient with active acromegaly(2), and in animal experiments, elevated values were also found in young rats and guinea pigs as compared with adults(3,4). A relationship between rate of growth and size of the soluble collagen pool has also been demonstrated in animals(5-8). The latter finding, taken together with the evidence previously cited, suggests that the level of excretion of urinary hydroxyproline peptides may be related to the size of the metabolically active pool of soluble collagen as previously proposed(1).

A striking increase in the amount of extractable, neutral salt soluble collagen has been demonstrated in several animal species following administration of lathyritic agents (9-13). This has occurred without detectable increase in the rate of synthesis of total collagen(10,13). In the light of this increase, the excretion of hydroxyproline has been measured in lathyritic animals in an effort to gain additional evidence for a relationship between amount of urinary hydroxyproline peptides and size of the soluble collagen pool under circumstances in which growth was not the primary factor in enlarging this pool. In the following experiments, simultaneous determinations of the neutral salt soluble collagen of the skin and urinary hydroxyproline have been made in lathyritic and control rats. A recent preliminary report(4) has demonstrated the presence of increased amounts of hydroxyproline in the urine of animals with lathyrism.

Experimental. Twenty-eight Sprague-Dawley rats (Holtzman) from 3 litters, delivered within 72 hours of each other, were weaned at

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19 days to a regular diet,‡ which on analysis contained 0.25% bound hydroxyproline. No significant difference in hydroxyproline excretion was observed when rats were fed the regular diet or a hydroxyproline-free artificial casein diet. After 4 days on the regular diet, the experiment was begun.

At random, 4 rats were set aside as baseline controls and the remaining individuals divided into 4 experimental and 4 control groups of 3 rats each. The groups were weighed, and experimental and control groups were then paired so that total weights of the animals in each pair did not differ by more than 5%. The groups were weighed daily, and during the second half of the experiment, food intake of the control groups was restricted when necessary to maintain their weights within 10% of the paired experimental groups.

Treated animals were injected daily during the first week with a solution of 30 mg of beta-aminopropionitrile (BAPN)§ in one ml of sterile water by the intraperitoneal route, and with 50 mg in one ml during the second and third weeks. BAPN solutions were made up just before injection. Control groups were injected with sterile water. Signs of lathyrism were apparent by the 18th day, as evidenced by gross deformation of the femurs on X-ray examination.

Pooled 24-hour urine samples from each group were collected twice weekly under toluene. To avoid contamination of the urine by food particles, individual groups were placed in metabolic cages during the collection period and fed 40 ml of a solution containing 20% dextrose, 0.3% NaCl and 0.4% KCl and tap water *ad libitum*. One experimental and matched control group were sacrificed with ether on days 7, 14, 18 and 21 immediately following the urine collection period. The abdominal skin was shaved, excised and carefully dissected free of fat. The pooled skin of each group was then divided into 2 weighed portions. One was finely minced immediately and extracted by shaking for 24 hours at 4° C. with 3 volumes (V/W) of 1 M NaCl containing 0.005 M phosphate at pH 7.6(10). Supernatants were obtained by centrifugation at 35,000 × g for 30 minutes at 4° C. The second portion of skin was dried to constant weight *in vacuo* over P_2O_5. Whole skin, extracts and urines were stored at −15°C.

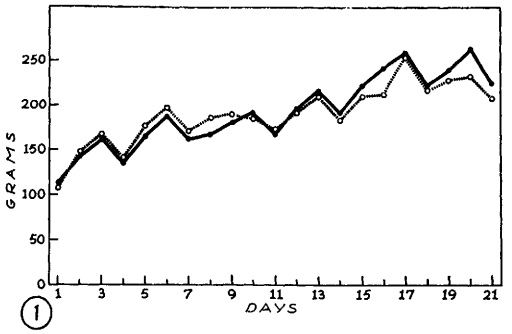
Urines and soluble collagen fractions were hydrolyzed in 6 N HCl for 3 hours in an autoclave at 18 to 20 lb. p.s.i. Whole skin was hydrolyzed for 15 hours under similar conditions. The hydrolysates were evaporated to dryness *in vacuo* over NaOH pellets at 60°C. The dry residues were then dissolved in distilled water, insoluble material discarded, and suitable aliquots taken for determination of hydroxyproline. Free hydroxyproline was determined on 2-ml samples of non-hydrolyzed urine. The zero time figures, both in experimental and control groups, represent the average of duplicate analyses of 10 pools of urine from 28 animals. The figures at 3 and 7 days represent averages of duplicate analyses of 8 pools from 24 animals; at 10 and 14 days, of 6 pools from 18 animals; at 18 days, analyses of 4 pools from 12 animals; and at 21 days, 2 pools from 6 animals.

Hydroxyproline was determined by the method of Prockop and Udenfriend(14) with the following modifications. To a 2-ml aliquot containing 2 to 10 μg of hydroxyproline, 2 g of solid KCl, 0.3 ml of a 10% alanine solution, pH 8.7, and 0.7 ml of 0.25 N borate buffer, pH 8.7, were added. Oxidation was carried out with 0.6 ml of 0.2 M Chloramine T in methyl cellosolve for 20 minutes at room temperature. The reaction was stopped by adding 2 ml of 3.6 M sodium thiosulfate. The samples were then extracted once with 3.3 ml of toluene and the toluene layer discarded. A second volume of 3.3 ml of toluene was then added and the mixture allowed to stand in a boiling water bath for 30 minutes. Color development was carried out using 1.7 ml of the toluene layer and 0.6 ml of paradimethylaminobenzaldehyde reagent. When hydroxyproline was determined on hydrolysates of whole skin or unhydrolyzed urine, the preliminary toluene extraction step was carried out twice. All analyses were done in duplicate.

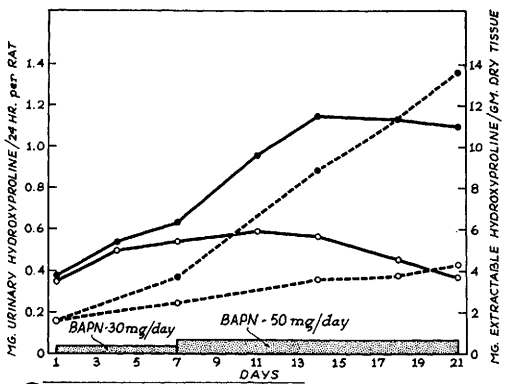
‡ Uncle Johnny Mills, Houston, Texas.

§ Supplied by Abbott Laboratories, North Chicago, Ill.

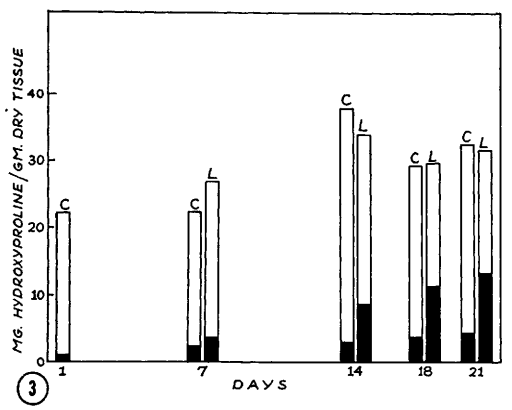
Results. Average daily weights for experimental and control animals are shown in Fig. 1. Maximum variation, which occurred on days 16 and 20, was approximately 15%. At the end of the third week, the surviving groups had doubled their weights. Dips in the weight curves correspond with restriction of food on the day of urine collection.



(1)



(2) Urine values represent average values from all surviving animals.



(3)

FIG. 1. Avg weights of groups. ⊞○⊞ control; —●— lathyritic.

FIG. 2. Total urinary hydroxyproline and soluble collagen of skin in lathyritic and control rats. —●— urinary hydroxyproline, BAPN treated; —○— urinary hydroxyproline, control; - -●- - soluble collagen, BAPN treated; - -○- - soluble collagen, control. Urine values represent averages obtained from surviving animals.

FIG. 3. Hydroxyproline content of skin. C, control; L, lathyritic; ■, extractable hydroxyproline; □, non-extractable hydroxyproline.

Fig. 2 shows average daily excretion of total hydroxyproline in the lathyritic and control groups in relation to the neutral salt soluble collagen of the skin. At 7 days, excretion of hydroxyproline in the lathyritic animals was approximately 10% greater, and extractable collagen of the skin 50% greater, than in the control groups. By the eleventh day, 24-hour urinary excretion values per rat had risen from 0.59 mg (0.49-0.69 mg) in the control group to 0.96 mg (0.94-0.99 mg) in the lathyritic group. This rise is statistically significant at the 0.01 probability level (as determined by the Student's t test). Three weeks after starting BAPN, the hydroxyproline excretion of the treated group was 1.1 mg, or 3 times, that of the control value of 0.37 mg. At the same time, the soluble collagen had similarly increased to approximately 3 times that of the corresponding controls. That the increased hydroxyproline in the urine was mainly peptide is indicated by the finding (Table I) that approximately 90% of the increase, as measured on day 18, was represented by bound hydroxyproline.

No significant difference in total hydroxyproline content of whole skin was demonstrated between experimental and control groups (Fig. 3). Maximum variation of the water content of the skin between paired groups was 2.2%.

Discussion. The total collagen content of the skin of the rats with lathyrism was not significantly different from that of the controls. This has been previously demonstrated in chick embryo skin(10) and in sponge implants(13) in lathyritic animals. The soluble collagen fraction was increased, however, and parallel with this increase, a rising excretion of urinary hydroxyproline peptides was observed. This finding provides additional evidence that the amounts of hydroxyproline peptides in the urine are a measure of the soluble collagen pool, which is known to be metabolically ac-

TABLE I. Free and Total Hydroxyproline in Urine of Lathyritic and Control Animals (18th Day).

	No. of rats	Total hydroxyproline (μg/24 hr)	Free hydroxyproline μg/24 hr	Free hydroxyproline % total
BAPN treated	6	1138	93.8	8.2
Controls	6	455	22.2	4.9
Increase with BAPN treatment	—	683	71.6	10.5

tive(15,16) and, therefore, presumably available for breakdown(1,17).

In contrast to previous observations demonstrating increased excretion of hydroxyproline in association with rapid growth(1,3), the present results in lathyritic animals have shown an increased excretion above that of controls growing at the same rate. Nevertheless, it cannot be concluded that the effects of BAPN are entirely independent of the growth phenomenon since there is evidence that young rats are more susceptible to lathyrism than adult animals(18,19). It is not unlikely, for this reason, that growth is in some manner permissive for the action of lathyrogenic agents. Selye and Bois(20) have, in fact, demonstrated a synergistic effect of growth hormone and aminoacetonitrile in development of lathyrism. It appears reasonable, therefore, to suggest that lathyrogenic agents increase the soluble collagen fraction by blocking the formation of fibrous collagen and that for this reason the effect of these agents is more easily demonstrable in the growing animal, where the rate of formation of soluble collagen is high. Though Schmidt and Orbison(21) have reported production of lathyrism in adult rats, the changes produced have been limited to bone where the turnover of collagen is known to be high even in the adult animal(22).

In view of the finding by Gross(5) of a rapid change in amount of extractable collagen with variation in weight, care was taken to maintain a similar rate of growth in the experimental and control groups. At no time did the weights of the paired experimental and control groups differ by more than 15%.

Martin, Mergenhagen and Prockop(4) have reported an elevation in hydroxyproline excretion in rats injected with 160 mg of BAPN daily. This elevation was detectable only between the 20th and 55th day, even though the rats were injected with over 3 times the dose of BAPN given in the present experiment. Since these authors did not determine soluble collagen, this discrepancy is not immediately explainable. It is possible that the low-protein diet employed in their experiments resulted in a slowing of the growth rate of their animals, thus rendering them to some extent refractory to the lathyrogenic agent.

Summary. Urinary excretion of hydroxyproline of weanling rats, rendered lathyritic by daily injection of beta-aminopropionitrile, was 3 times greater than that of controls. A parallel increase in the neutral salt extractable collagen of the skin was observed. No significant change was found in the total collagen of the skin. It is concluded that the increase in urinary hydroxyproline observed is related to the increased size of the metabolically active, soluble collagen pool.

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Mumps III. Comparison of Methods for Detection of Viruria. (27666)

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In rhesus monkey kidney cell cultures inoculated with specimens containing mumps virus a distinctive cytopathic effect (CPE) appears(1), and the infected tissue culture cells adsorb erythrocytes (hemadsorption–HAD)(2). The following investigation was undertaken to compare these newer technics with the older methods of hemagglutination (HAG) and embryonated hen's egg inoculation in detection of virus in urine.

Methods. Patients. Patients with suspected mumps were studied while hospitalized at the Clinical Center Nat. Inst. of Health, at the Nat. Naval Med. Center in Bethesda, or in a few instances while at home. Days of illness were numbered in relation to the first day the patient observed parotid or submandibular gland swelling or pain.

Specimens. Since virus can be detected in urine for a considerably longer period than in oral or throat swabs(3) urine specimens were studied. The first voided morning specimen was collected in a sterile container, stored at 4°C and delivered to the laboratory within 2 to 5 hours. In a few instances when the specimen was inadvertently left at room temperature, a later morning urine was collected and both specimens were studied. Urines were collected every Monday, Wednesday and Friday while the patient was under observation.

Specimens were prepared by ultracentrifugation as previously described(3) and the pellet was suspended in medium 199 with 0.5% gelatin. The prepared specimens were immediately inoculated into cell cultures and embryonated hen's eggs.

Blood was collected from most patients on the first and last day of observation.

Cell cultures. Rhesus monkey kidney cell cultures were used within 7 days of receipt in the laboratory. They were inoculated with 0.1 ml of the prepared specimen and observed daily thereafter. Fluids were passaged at least once at the time when CPE was judged maximal or otherwise 13-14 days after inoculation.

Hemadsorption. At the time of passage or discard the supernatant fluids were removed and 0.3 ml of a 0.4% guinea pig or human group O erythrocyte suspension in saline was added to the cell culture tubes. After standing for 20 minutes at 4°C, the tubes were observed microscopically for hemadsorption. Controls of uninoculated monkey kidney cell cultures were included.

Hemagglutination. Serial 2-fold dilutions of the cell culture supernatant fluids were prepared in 0.5-ml amounts of 0.9% saline solution. An equal volume of a 0.4% suspension of guinea pig erythrocytes was added and the tubes shaken. After the tubes had remained standing at room temperature for 90 minutes the highest dilution showing hemagglutination was determined.

Hemagglutination-inhibition. Control and anti-mumps guinea pig or rabbit sera(1) were