strain of serotype O_2K_1 contained sialic acid in bound form. This sialic acid could be released by mild hydrolysis, as verified by the thiobarbituric acid test which measures only free sialic acid, and as confirmed by paper chromatography. Aliquots of such lysates, containing 30 to 50 μg of N-acetylneuraminic acid in bound form, injected intraperitoneally in mice caused toxic symptoms including anorexia, loss of weight, lethargy and occasionally death. However, comparable lysates of 24 hour old cells of the same strain, which contained no sialic acid, caused the same symptoms when injected in mice. Further when injected intradermally in combination with epinephrine in the shaved abdomen of rabbits, both cell-free preparations produced hemorrhagic lesions at sites of the injections. These results indicated that sialic acid played no essential role in the toxicity of these cell extracts.

Discussion. The data tabulated in Table I show that there is no correlation between sialic acid content of cells of strains of *E. coli* and lethality for mice. Increased lethality must depend on survival of a small inoculum in the host and it is during the lag phase that the inoculum would be expected to be most vulnerable to host defense mechanisms. The 24 hour old cells which contained little, if any, sialic acid were as lethal and hence as able to survive in the host as the 5 hour cells which contained as much as 1 to 1.5% sialic acid (Table II). Hence, sialic acid does not play an essential role in protecting the cells during their lag phase from the host defense mechanism.

Summary. The lethality of 13 *E. coli* strains, each of a different serological type, was compared in mice and was found to differ greatly. These differences could not be attributed to differences in heat stable toxins nor to presence or absence of sialic acid in the cells. *E. coli* strains containing sialic acid gradually lost most, if not all, of this monosaccharide after 24 hours growth in culture, but no corresponding decrease in lethality occurred. Cell-free lysates of *E. coli* containing bound sialic acid were toxic for mice, and when injected in combination with epinephrine produced hemorrhagic lesions in rabbits, but no more so than did comparable lysates from older cells of the same strain which contained no sialic acid.

The technical assistance of Mrs. Helen N. Dilts is gratefully acknowledged.

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Received June 6, 1961. P.S.E.B.M., 1961, v108.

Dietary Composition and Tissue Protein Synthesis I. Effect of Tryptophan Deficiency.* (26838)

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Protein deficiency is a well known factor in growth retardation. A low dietary level of an adequate protein or of one of the essential amino acids in an otherwise complete diet is the most common way of inducing a protein deficiency. Protein free diets or com-

* This investigation was supported by U.S.P.H.S. grants.

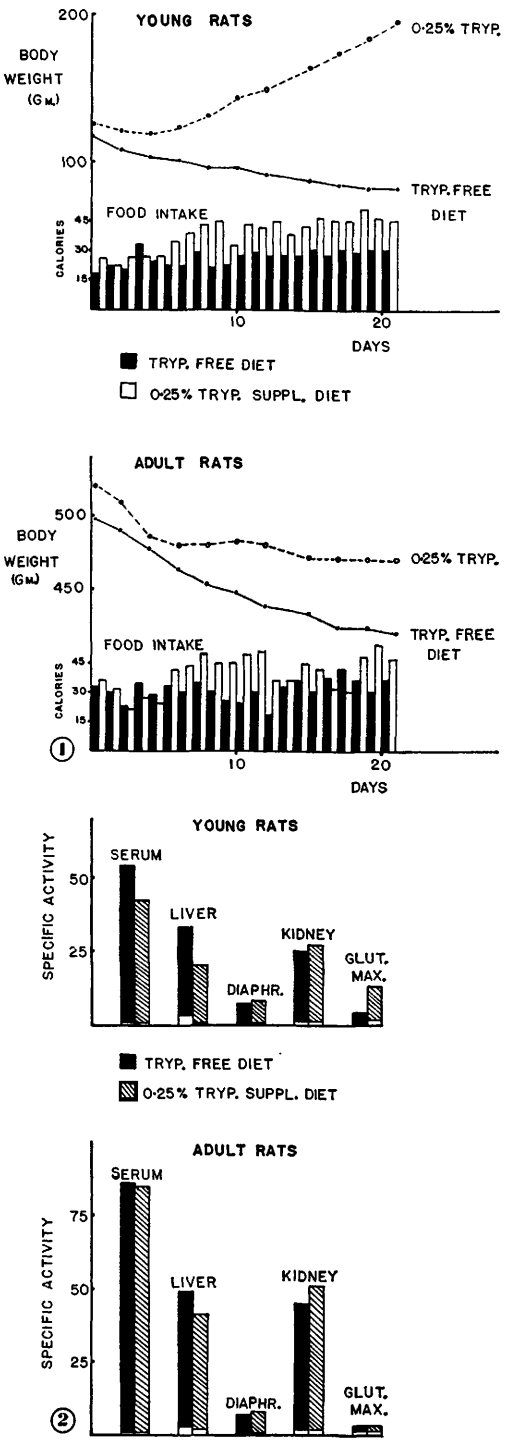

FIG. 1. Body wt changes and food intake of young and adult male rats fed a tryptophan-free diet and a diet supplemented with 0.25% tryptophan.

FIG. 2. Specific activities of tissue proteins of rats fed a tryptophan-free and a supplemented diet. Rats sacrificed on 13th day of experiment, 5 hr after inj. of 1-C^{14}-glycine. White area below each column represents non-protein fraction specific activity.

plete absence of an essential amino acid are the extreme experimental conditions often encountered. The loss of body and organ weight under these circumstances is well established as is the fact that not all organs are depleted at the same rate(1). It was our purpose to study different tissues as to their ability to synthesize protein as measured by uptake of injected C^{14}-glycine. Of special interest was the metabolism of skin and newly forming connective tissue such as that which is stimulated by subcutaneous implantation of plastic surgical sponge.

Methods. Young and adult male rats of the Holtzman strain were implanted subcutaneously with sterile polyvinyl sponges (Ivalon) and placed on tryptophan free 24% acid casein hydrolysate† and tryptophan supplemented diets(2). On the 13th day of experiment all rats were injected intramuscularly with 1-C^{14}-glycine (4.5 mc/mM) in saline solution. The adult rats received 1 μc (7.3×10^5 counts per minute) per 100 g of body weight and the young animals 3 μc (2.19×10^6 counts per minute) per 100 g of body weight. Because of the different levels of radioactivity injected relative values rather than absolute values should be compared among the different age groups.

One group of animals was sacrificed on the 13th day, 5 hours after receiving the labeled glycine. The other group was sacrificed on the 21st day of experiment, *i.e.*, 8 days following isotope administration. Sacrifice was performed by overexposure of the animals to ether. Skin, sponge, and the tissues under study were removed and protein and non-protein fractions and collagen isolated and counted as previously described(3). Collagen was determined by a modification of the Neuman and Logan method for hydroxyproline(4) after the samples had been hydrolyzed in 6 N HCl at 25 p.s.i. during 3 hours.

† Salt Free Hydrolysed Casein, Sheffield Chemical, Division of National Dairy Products Corp., Norwich, N. Y.

All values for tissue protein radioactivity are expressed as specific activity (counts/minute/mg tissue protein or collagen/μc glycine-1-C^{14}).

Results. Changes in body weight of the different experimental groups are shown in Fig. 1. Young animals fed the tryptophan supplemented diet gained an average of 3.3 g per day. The non-supplemented group lost weight.

Neither diet when fed to adult rats was able to support body weight, although the tryptophan supplemented diet was more efficient in counteracting weight loss.

Collagen contents of skin and subcutaneously implanted polyvinyl sponge and percent of dry fat-free solids of the skin of the young animals 13 and 21 days after initiation of the experiment are shown in Table I. Values for dry fat-free skin solids were greater in the deficient animals than in the supplemented group at both the 13th and 21st day of experiment. A similar increase in skin collagen is also evident in the deficient group as compared to the supplemented control. This does not mean increased synthesis but probably a change in the relative composition of skin. However, sponge collagen was significantly increased in those animals fed the complete diets, thus indicating that formation of new connective tissue proceeds at a faster rate during the feeding of an adequate diet.

The specific activity of collagen isolated from skin and sponge of these animals was also determined. These values were meant to furnish an idea of the rate of collagen synthesis at the different sites.

Thus the rate of collagen synthesis in the skin, as judged by specific activity, occurring in the tryptophan supplemented rat is 4 times that observed in the unsupplemented animal. In direct contrast to the skin, no significant differences are to be observed in the newly formed sponge collagen. The slightly decreased specific activity of sponge collagen in Group IV could be accounted for by the larger amount of collagen present at the end of the 21st day, causing an isotopic dilution which followed the peak of maximum glycine-C^{14} incorporation.

It seemed of interest to calculate the ratio of sponge to skin collagen specific activities. This ratio could be interpreted as an index of the synthesis of new collagen in response to the stress of a sponge implant as compared to its synthesis in the skin.

In the tryptophan supplemented animals

TABLE I. Total Content and Specific Activity of Skin and Subcutaneously Implanted Sponge Collagen of Young Growing Animals Fed a Supplemented and Unsupplemented Tryptophan-Free Diet for Varying Periods.

Group	Supplement	Exp. period (days)	% dry fat-free skin solids	Skin collagen (% wet wt)	Total sponge collagen synthesized (mg)	Time after isotope inj.	Collagen specific activity†		Ratio, sp./skin
							Skin	Sponge	
I	None	13	24.7	13.9 ± 1.1*	10.1 ± .8	5 hr	12 ± 2.3	56 ± 6	4.7
II	.25% try.	13	21.8	9.5 ± .7	13.2 ± .3	5 "	49 ± 11.0	60 ± 2	1.2
III	None	21	27.0	13.8 ± .5	15.2 ± .9	8 days	8.4 ± 1	58 ± 12	6.9
IV	.25% try.	21	22.7	11.2 ± .9	22.0 ± .9	8 "	23.3 ± 2	46 ± 6	2.0

* Stand. error.

† Counts/min./mg collagen μc glycine-1-C^{14}/100 g body wt.

the ratio was close to 1 (Group II) indicating that collagen synthesis proceeded at a constant rate throughout the body at that time. In Group IV isotope dilution would again account for a slight increase in ratio 8 days following tracer injection. This ratio was increased in the deficient rats indicating a sparing effect which would favor new tissue formation as a response to sponge implant during inadequate nutrition.

TABLE II. Total Content and Specific Activity of Skin and Subcutaneously Implanted Sponge Collagen of Old Rats Fed a Supplemented and Unsupplemented Tryptophan-Free Diet for Varying Periods.

Group	Supplement	Exp. period (days)	% dry fat-free skin solids	Skin collagen (% wet wt)	Total sponge collagen synthesized (mg)	Time after isotope inj.	Collagen specific activity			
							Skin	Sponge	Ratio, sp./skin
V	None	13	29.2	17.6 ± .5	11.6 ± 1.3	5 hr	7 ± .4	83 ± .9	11.8
VI	.25% try.	13	28.5	17.4 ± .5	12.6 ± .8	5 "	9 ± .9	100 ± 4.1	11.1
VII	None	21	30.9	17.9 ± .5	15.8 ± .7	8 days	8.3 ± 1	113 ± 10	13.6
VIII	.25% try.	21	28.7	16.8 ± .1	14.9 ± .9	8 "	9.3 ± .6	106 ± 11	11.4

Results of the experiments in older adult rats are shown in Table II. Tryptophan deficiency slightly increased the percentage of dry fat-free skin solids. Skin collagen values were not significantly altered by the nature of the diet. They were elevated in all groups with respect to the younger animals. Sponge collagen determinations failed to show significant differences in net synthesis between tryptophan supplemented and unsupplemented groups.

Table II summarizes specific activity determinations of the collagen samples isolated from adult rats. Skin as well as sponge collagen values in the tryptophan supplemented group were slightly higher after 5 hours post injection of C^{14}-glycine.

Because of the slow rate at which skin collagen is synthesized in the adult rat, the isotopic dilution effect was not observed in these animals. The sponge/skin ratio was almost constant. These results seem to indicate that in adult animals dietary imbalance does not cause the marked changes in rate of collagen synthesis that are observed in younger animals. However, body weight loss occurred in all adult rats regardless of presence or absence of tryptophan in the diet.

Fig. 2 summarizes results obtained when rate of labeling of different tissue proteins other than collagen were studied in young and adult rats receiving the tryptophan deficient and supplemented diets over a 13-day period. The specific activities of tissue proteins 5 hours after injection of 1-C^{14}-glycine are compared. Non-protein tissue radioactivity is represented by the white area below each column.

The marked differences in growth observed in young animals lacking tryptophan in the diet were not reflected in the rate of tissue protein labeling. Serum and liver proteins of the young deficient animals had a higher specific activity than that of the supplemented ones. On the other hand the gluteus muscle of the tryptophan supplemented rats incorporated the labeled glycine more efficiently.

The radioactivity of the non-protein moi-

ety was increased in those organs where protein synthesis was enhanced. Liver and gluteus muscle showed the most significant increase of this fraction. This agrees with the findings of Clark(5) and our observations on the stimulating effect of testosterone propionate on different tissues in which an increased rate of protein synthesis was always accompanied by a concomitant increase in non-protein nitrogen or radioactivity of this fraction (3).

A similar pattern was observed in adult animals, though no increased labeling was apparent in the gluteus muscle.

Discussion. Both age and tryptophan deficiency seemed to increase the amount of fat-free solids in the skin. Also the relative amount of skin collagen present was increased by the dietary deficiency in growing animals. Adult rats all showed a greater collagen content than young rats, independent of the nature of the diet. These changes in collagen content should not be equated to a net synthesis but rather to a change in relative composition of skin. Fat and water content of the skin are markedly influenced by the nutritional state and can be responsible for changes in total protein content of the skin(6). Also the non-collagenous skin proteins are more readily depleted by nutritional deficiency than the collagen fibers, which have a much slower turnover rate. The specific activity of skin collagen isolated 5 hours after injection of C^{14}-glycine was 4 times greater in young animals receiving the complete diet than in the deficient controls. In spite of the lower relative amount of collagen present in skin the rate of synthesis was far more active in these animals. This agrees with the fact that the supplemented animals were growing whereas the deficient were losing weight.

Total collagen synthesized around the implanted sponge was greater in the young animals receiving tryptophan as a dietary supplement. No such difference was observed in older rats receiving the same supplement. A decreased collagen content of healing wounds and of subcutaneously implanted sponge biopsies of protein depleted rats has been demonstrated(7,8).

Nevertheless rate of synthesis of sponge collagen did not vary significantly with the nature of the diet as occurred with the skin. A ratio of sponge/skin specific activities was calculated, to serve as an index of how the organism favors collagen synthesis at different levels as a consequence of variables in diet and age. Older animals under all circumstances greatly favored synthesis of collagen at the sponge site. In these animals the diet had no apparent effect and did not influence the balance in rate of synthesis between sponge and skin.

The surprising fact that rate of uptake of labeled glycine by several tissues studied was not reduced by lack of tryptophan in the diet, but increased in serum proteins and liver suggests that at least at these sites protein synthesis occurs normally or, as seems apparent, at an increased rate. Similar findings have been reported by Sidransky and Farber(9) who found that rats fed a threonine deficient diet incorporated several radioactive amino acids into liver and serum proteins at a faster rate than rats fed supplemented diets. Waterlow(10) feeding rats a low protein diet found that protein-depleted animals incorporated S^{35}-methionine into the visceral organs (including liver) at a faster rate than the normal controls. In spite of this increased labeling during protein deficiency all animals consistently lost weight. The liver, where protein synthesis would seem to be most enhanced by nutritional deficiency is, however, one of the most rapidly depleted organs(1). This would suggest that increased protein synthesis occurs simultaneously with an even greater increased protein catabolism. The stimulation of protein synthesis could be the cellular response to the enhanced catabolic state.

The possibility that this mechanism is under hormonal control is being studied. The possibility that dietary deficiency (amino acid deficiency, amino acid imbalance, etc.) may cause increased mobilization of protein stores of the body should be considered as a likely cause of turnover stimulation at such sites as liver and serum.

Summary. Rate of collagen synthesis, as measured by 1-C^{14}-glycine uptake, by skin

and in subcutaneously implanted polyvinyl sponge has been shown to vary with age and diet. A tryptophan-deficient diet significantly reduced the rate of skin collagen synthesis in young animals. However, the greater loss of more labile components of the skin resulted in a relative greater percentage of skin collagen in this group. Rate of synthesis in the sponge was not significantly affected. The deficient animals showed an increased specific activity in liver and serum proteins, suggesting that protein synthesis probably occurs at a faster rate in the liver simultaneously with increased catabolism or utilization.

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Received June 9, 1961. P.S.E.B.M., 1961, v108.

Identification of 24-Dehydrocholesterol in Human Blood Vessels Following MER-29 Therapy.* (26839)

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Avigan *et al.*(1) have demonstrated that inhibition of sterol biosynthesis by MER-29 (Triparanol) results in accumulation of 24 dehydrocholesterol in rat livers. 24-dehydrocholesterol has been identified by Steinberg *et al.*(2) in the blood of humans given MER-29. Little is known of the biologic activity of 24-dehydrocholesterol in man except that it does not appear spontaneously in human blood(3). The use of MER-29 for treatment of hypercholesterolemia in man makes it important to learn if 24 dehydrocholesterol accumulates in blood vessel walls as well as in blood.

Materials and methods. Vessels were obtained at autopsy from a 16-year-old white male whose diagnoses were: familial hypercholesterolemia, congenital aortic stenosis and recent myocardial infarction. The patient had received 250 mg per day of MER-29 for 79 days. Extensive atheromatosis covered 90% of the area of the aortic wall. Specimens of normal vessel wall were taken from between atheromas and stripped of adventitia. Atheromas were graded(4), freed from surrounding tissue, and stripped of adventitia.

Serum was saponified and extracted with petroleum ether as described by Abell *et al.* (5). Tissue specimens were saponified in 20% ethanolic KOH and extracted with one volume of water and 2 volumes of petroleum ether. Petroleum ether residues were taken up in chloroform for gas liquid chromatography (GLC).

GLC was performed in ⅛ inch glass columns, packed with Gas Chrom P† coated with neopentyl adipate terminated (NAT), neopentyl glycol sebacate (NGS) as described by Lipsky(6), or QF-1 (10,000 C.S.) as described by VandenHeuvel *et al.*(7). An argon ionization detector containing radium was used and was calibrated with a known mixture of cholesterol and 24-dehydrocholes-

* This work was supported by grants from USPHS.

† Applied Science Laboratories, Inc., State College, Pa.