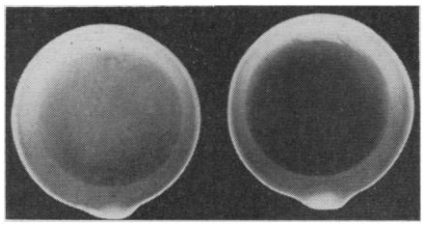

Fig. 1. Melanin formation in blood of *Philosamia cynthia ricini*. (Left) Retarded melanin formation by allithiamine; (right) normal melanin formation.

duced: allyl mercapto radical (CH_2=CH—CH_2—S·) and thiaminyl thiol [(B_1)—SH]. The former is well known to combine with sulfhydryl (SH) groups in vivo (*2, 4, 8*), so that it is probably the latter which will combine with cupric tyrosinase according to the following equation:

$$(B_1)\text{-S-S-}CH_2\text{-CH=}CH_2 + R\text{-SH} \xrightarrow{\text{(metal enzyme)}}$$

allithiamine; SH group in blood

$$R\text{-S-S-}CH_2\text{-CH=}CH_2 + (\text{Metal enzyme})\text{-S-}(B_1)$$

allyl disulfide; thiaminyl mercaptide

This interpretation of the above results may be applicable to the reaction between allithiamine and other metal enzymes in vitro as well as in vivo (*9*).

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4 December 1957

Effect of Light on Fluorescence of Ethylenediamine Derivatives of Epinephrine and Norepinephrine

In 1953 Weil-Malherbe and Bone (*1*) described a procedure for the differential estimation of epinephrine and norepinephrine in mixtures. They found that, after incubation with ethylenediamine, the fluorescence of these two compounds differed enough so that at 500 mμ the ratio of the fluorescence of epinephrine to norepinephrine was 0.98 whereas at 550 mμ it was 4.4. The solutions appeared to be stable for 24 hours at room temperature under ordinary light conditions. Persky and Roston (*2*) published curves representing the emission spectra of these substances which were in substantial agreement with these findings.

Studies conducted by Aronow and Howard (*3*) and studies made in our own laboratory (*4*) suggested that entirely different ratios were obtained when the procedure was carried out in a room illuminated by red or orange light. The difference appeared to be due to changes in the intensity and distribution of the norepinephrine fluorescence. Crude experiments performed at that time indicated that about 80 percent of the fluorescence of the norepinephrine was lost if the reaction was carried out in daylight. When daylight was excluded, the ratio of epinephrine to norepinephrine at 485 mμ was approximately 0.2 and at wavelengths above 550 it was about 3.2, when light at a wavelength of 436 mμ was used to activate the solutions. The effect of blue light was also evidenced by the decay during activation at 436 mμ in the Farrand photoelectric fluorometer, as noted by Valk and Price (*5*) and Mangan and Mason (*6*).

Mangan and Mason (*6*) have recently published curves for the fluorescent products of the above reaction which differ from those of Persky and Roston (*2*). It is the purpose of this report (*7*) to suggest that the differences described may be related to quantitative or qualitative differences, or both, in the exposure to light and, furthermore, that the exclusion of blue and ultraviolet light results in an emission spectrum differing from the previously published findings.

Measurements were made with the Aminco-Bowman spectrophotofluorometer. In order to obtain the entire emission spectrum between 400 and 600 mμ without interference from scatter by the source of activating light, high concentrations of the amines were employed. However, experiments with the concentrations employed by Mangan and Mason produced similar results except for the presence of a significant blank at the lower concentrations.

Duplicate samples of epinephrine and norepinephrine (*8*) were prepared in alumina-treated acetic acid. One of the samples of each amine was incubated with ethylenediamine and ethylenediamine dihydrochloride for 40 minutes at 50°C. After saturation with sodium chloride, the solutions were extracted with isobutanol. The final extract contained the equivalent of 12 μg of epinephrine or norepinephrine per milliliter. The entire procedure was carried out in a room illuminated by daylight. The duplicates were carried through the same procedure in a room illuminated only by a 25-watt ruby darkroom lamp.

Samples were read at intervals of 5 mμ on the spectrophotofluorometer, and the galvanometer readings are illustrated in Fig. 1. The spectra were obtained by irradiating the samples at the wavelengths producing maximum activation; this area was found to lie between 420 and 425 mμ for norepinephrine and between 430 and 435 mμ for epinephrine. When both solutions were activated at 436 mμ, the relationship illustrated was slightly altered inasmuch as the relative fluorescence of norepinephrine was reduced by 7 percent. After the initial reading was taken of those solutions that were prepared in the darkroom, the cuvettes were exposed to daylight for 5 minutes in order to reduce the fluorescence of the norepinephrine sample to approximately 50 percent. The results of these experiments are illustrated in Fig. 1.

In Fig. 1, the upper left-hand curves were obtained from the solutions carried through the procedure in the dark. The middle curves on the left were obtained by rereading the same solutions after exposing them to daylight for 5 minutes. The lower curves on the left represent the galvanometer readings on the duplicates of the above samples which were carried through the procedure without excluding daylight. The similarity of the curves obtained from the second and third experiments to those of Mangan and Mason and of Persky and Roston is apparent. It can also be seen that daylight, in addition to causing a loss of fluorescence of norepinephrine, causes a small shift in the fluorescent maxima.

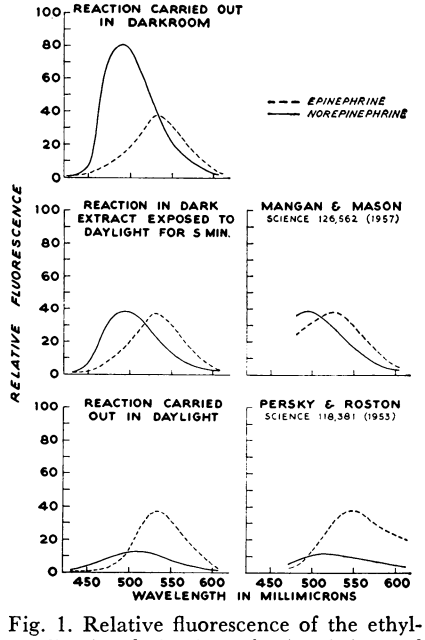

Fig. 1. Relative fluorescence of the ethylenediamine derivatives of epinephrine and norepinephrine.

Little effect on the fluorescence of epinephrine was noted.

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Digestibility of Uniformly Labeled Carbon-14 Soybean Cellulose in the Rat

Reports in the literature on the fate of ingested cellulose in nonruminant animals and man present a confusing picture. Some workers (*1*) state that digestion, or utilization, of cellulose takes place to an extent varying from 1.5 to 97 percent of the ingested cellulose, while others (*2*) conclude that cellulose cannot be utilized at all. The present isotope study was undertaken in an attempt to resolve these discrepancies.

Uniformly labeled C^{14}-cellulose was prepared from defatted C^{14}-labeled soybean meal. Protein was removed from the meal by thorough extraction, first with cold water, then with 10-percent aqueous sodium hydroxide. Hemicelluloses were further extracted with 10-percent aqueous potassium hydroxide, and the residue was washed successively with water, ethanol, and ether. The dried product (specific activity, 9060 count/min mg) was used in the feeding experiment. This material contained less than 1 percent of protein and yielded no reducing sugars after 6 hours' hydrolysis in 2*N* sulfuric acid at 95°C. Since cellulose is the only major non-nitrogenous constituent present in defatted soybean meal which shows these solubility characteristics and this resistance to acid hydrolysis, the material was considered to be essentially pure cellulose.

Four growing male albino rats (Sprague-Dawley), weighing from 120 to 180 g, were selected for the feeding experiment. During the 4 days preceding the administration of labeled cellulose, the rats were fed by stomach tube a diet consisting of casein (20 percent), starch (61.5 percent), Wesson oil (10 percent), cellulose (4 percent), minerals (4 percent), and vitamins (0.5 percent). This diet was mixed with water in a ratio of 1:6 by weight to facilitate the stomach-tube feeding and was supplemented by a standard diet and water ad libitum.

Table 1. Recovery of radioactivity from uniformly labeled C^{14} soybean cellulose fed to rats.

Rat No.	Time in metabolism cage (hr)	Recovery of C^{14} (%)						
		In urine	In feces			In carcass	In expired CO_2	Total
			Petroleum ether insoluble	Petroleum ether soluble (by difference)	Total			
1	79	1.0	47.1	0.1	47.2	19.6	40.4	108.2
2	79	0.4	37.8	10.5	48.3		43.9	
3	96	1.1	42.9	1.6	44.5	9.3	57.0	111.9
4	100	2.3	39.8	13.0	52.8	7.9	45.6	108.6

Following the conditioning period, the rats were placed in metabolism cages attached to gas trains for collection of urine, feces, and expired CO_2. The above diet, with C^{14}-labeled cellulose in place of the nonradioactive cellulose, was fed by stomach tube 3 times daily; a total of 15 to 17 g of the diet-water mixture was thus administered each day for 3 days. A carmine marker mixed with the final C^{14}-cellulose feeding marked the final excretion in the feces of labeled material.

Urine, feces, and expired CO_2 were collected for each rat during the time spent in the metabolism cages. Immediately upon removal from the metabolism cages, the rats were sacrificed, with ether, and the carcasses, plus viscera, were frozen in liquid nitrogen and ground in the frozen state in a burr mill to obtain a carcass homogenate.

All of the homogenized, dried samples, with the exception of urine, were analyzed for C^{14} content by combusting to CO_2 and counting the labeled CO_2 as precipitated barium carbonate. After the dried feces were counted, this material was extracted by the Soxhlet method for 24 hours with petroleum ether (40 to 60° fraction) to remove the lipid portion. The fat-free residues were dried and analyzed for C^{14} content. The total C^{14} in feces, the petroleum-ether insoluble C^{14} in feces, and the petroleum-ether soluble C^{14} in feces (by difference) are recorded in Table 1. An aliquot portion of each urine sample was plated at infinite thinness and counted directly. All calculations were corrected for ash content of the cellulose.

Data tabulated in Table 1 indicate that a large proportion of the ingested cellulose is converted in the gastrointestinal tract to a form which the rat absorbs and metabolizes readily. The data show that only half of the C^{14} fed the rat is excreted in the feces, the rest being found distributed in the expired CO_2, carcass, and urine.

Since significant percentages of the C^{14} in the feces are present in the form of petroleum-ether soluble materials, it is apparent that the ingested cellulose, which is completely insoluble in petroleum ether, is altered as it passes through the rat's gastrointestinal tract. This finding is in agreement with work reported by others (*3*), who find that a number of microorganisms isolated from the gastrointestinal tract are able to convert cellulose, to a greater or lesser extent, to fatty acids and other metabolic products. It is quite possible that these petroleum ether extractives are among the types of cellulose degradation products which are absorbed into the rat tissues. If this is the case, it seems likely that the production of the absorbable materials from cellulose is a result of intestinal bacterial action rather than of the action of enzymes arising in the rat's own digestive juices.

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