lens derived from the young rat (aged 28 to 32 days) is 5.69 as compared with a DPNH:DPN ratio of 1.52. (*2*) These results provide further evidence of an active shunt pathway in this organ.

There must be some mechanism within the lens to oxidize the relatively large amounts of TPNH formed during the first two steps of glucose oxidation via the shunt. Employing the method of Kaplan *et al.* (*3*) and Stein *et al.* (*4*) in which DPN analogues are used, I was unable to demonstrate by repeated assays any transhydrogenase activity within the rat lens. Although a relatively inactive TPNH-linked lactic dehydrogenase is present in this organ, its activity is only approximately 1/15 the activity of DPN lactic dehydrogenase (*5*) at *p*H 7.4, and it is difficult to see how such a system could account for an efficient and rapid reoxidation of TPNH.

TPNH and DPNH cytochrome *c* dehydrogenase were then looked for in the lens. Pirie *et al.* (*6*) have reported that TPNH cytochrome *c* dehydrogenase activity is present in the rat lens. Employing their assay system, I discovered that cytochrome *c* could be reduced very rapidly if only an aliquot of a lens homogenate—and no TPNH or DPNH—was added to the cuvette. Four lenses derived from 28- to 32-day-old Holtzman strain white male rats were therefore homogenized in 5 to 10 times their weight in deionized water, dialized overnight, and centrifuged; and the supernatant solution was retained for the assay procedure. Since an aliquot of this supernatant solution was also capable of rapidly reducing cytochrome *c* (without any added DPNH or TPNH), the solutions were assayed for any ascorbate that might still be present in a bound form. The Roe procedure based on the reduction of the dye 2,6 dichlorophenolindophenol (*7*) was employed as a simple assay for ascorbate, and the results showed that no ascorbate was present in these dialized solutions. There were also no sulfhydryl groups present (for example, reduced glutathione or cysteine) in sufficient quantity to account for the degree of cytochrome *c* reduction. Heating the solution for 2 minutes at 100°C completely destroyed its activity. Studies are now in progress to further characterize and determine this compound.

In order to assay for TPNH and DPNH cytochrome *c* activity, it was thus necessary to add a large excess of cytochrome *c* (type III Sigma) to the test system and allow the initial reduction of cytochrome *c* to go to completion. Approximately 30 to 45 seconds were required for this to occur. Either TPNH or DPNH was then added to the cuvette, and further reduction of cytochrome *c* was determined for a 5-minute interval. The results of such assays on four samples obtained from 16 rat lenses for TPNH and four samples (16 lenses) for DPNH cytochrome *c* reductase activity are shown in Table 1. Both TPNH and DPNH cytochrome *c* reductases are present within the rat lens, and the former is apparently about 30 percent more active (*8*).

Table 1. Results of assays for TPNH and DPNH cytochrome *c* reductase activity. The enzyme activity is expressed as the change in optical density at 550 mμ of 0.001 per minute at 24°C per lens.

Lenses (No.)	Av. wet weight per lens (mg)	Cytochrome *c* reductase activity	
		TPNH	DPNH
4	19.10	14.40	6.91
4	19.20	17.75	14.40
4	19.02	19.72	14.79
4	19.60	19.72	14.709
Mean activity		17.90	12.72

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Estimation of Total Body Fat from Potassium-40 Content

Abstract. On the assumption that the potassium content of the lean body mass is constant, it should be possible to estimate fat content in living man from a measurement of potassium-40 activity in the whole-body scintillation counter. A series of such measurements on children and young adults shows good correlations with skin-fold thickness and weight/height ratio as indices of fatness.

Current interest in obesity has resulted in attempts to assess the actual fat content of the body in living human subjects. Methods now in use include calculations based on measurement of total body water and body density (*1*) as well as inferences drawn from measurements of skin-fold thickness. The assumptions involved in the first two methods, and their inherent limitations, have recently been discussed (*2*).

The purpose of our report is to suggest a new approach to this problem, namely the use of whole-body potassium content as an index of lean body mass. This approach is based on results of chemical analyses of adult human subjects; only four such analyses have been done, and these revealed values of 66.5, 66.6, 72.8, and 66.8 meq/kg of lean body weight (*3*) (this term is taken to mean body weight minus chemically determined neutral fat). Other workers have emphasized the relative constancy of whole-body potassium content in such species as the cat, rabbit, rat, and pig when values are expressed on a fat-free basis (*4*), though the absolute values are somewhat higher than those for man. There is a high correlation between K^{40} content and lean tissue mass in hams (*5*). Woodward *et al.* (*6*) found a good correspondence between K^{40} content and lean body mass as determined by tritium dilution in man. Meneely *et al.* (*7*) were able to correlate K^{40} activity with basal heat production. The assumption of a constant potassium content for the lean body mass would thus appear to be a reasonable one.

Fifty subjects were accordingly assayed for K^{40} in a whole-body scintillation counter, and the estimates of fat content so derived were correlated with skin-fold thickness and weight/height ratio.

The subjects comprised 42 males, aged 11 to 44 years, and 8 females, aged 7 to 23 years. Body weights ranged from 22 to 105 kg. All were judged to be in good physical health. An attempt was made to include both obese and thin subjects so the results cannot be taken as representative of the normal population. Subjects were dressed only in a light cotton gown and paper slippers at the time of measurement. Skin-fold thickness was determined at six locations using special calipers (*8*): mid-biceps, mid-triceps, abdomen below and 1 cm to left of umbilicus, subcostal at nipple line, iliac crest, and subscapular.

Potassium-40 measurements were made in a whole body scintillation counter, according to the technique described in detail by Miller (*9*). The subject reclines in a specially constructed metal chair and is viewed by an 8-inch diameter, 4-inch thick sodium iodide crystal. The gamma rays emitted by the subject, which penetrate the crystal, produce scintillations which are seen by four 3-inch photo multiplier tubes. The signal output from the pho-

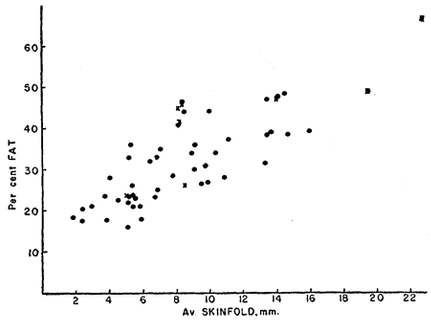

Fig. 1. Percentage of fat in body versus average skin-fold thickness. Females (x), males (•).

tomultipliers goes to the spectrum analyzer dropping into any one of 100 channels depending on the pulse amplitude. Potassium-40 is measured by counting the pulses in the photopeak for a measurement time of 30 minutes. Background interference is reduced by placing the subject and the crystal detector in a room with 8-inch thick steel walls. The system has been calibrated by the measurement of laboratory personnel of a selected size range who ingested known amounts of potassium-42. The sensitivity and reproducibility are such that remeasurement of the same individual gives the same result with a standard deviation of about 1.5 percent. Stable potassium content can be readily calculated from the normal abundance of K^{40}.

Fat content was calculated as the difference between total weight and lean body weight (LBW), on the basis that the latter has a potassium content of 68.1 meq/kg:

$$\text{LBW (kg)} = \frac{\text{measured total K (meq)}}{68.1}$$

This equation is analogous to that in common use for determination of LBW by deuterium or tritium dilution: namely, LBW = total body water/720, where the denominator is the water content per kilogram of LBW.

The range of potassium content for our subjects was 35–58 meq/kg in the males, and 23–52 meq/kg in the females. These values compare favorably with those reported by Anderson and Langham (*10*) in a much larger group of subjects studied at Los Alamos. Calculated fat content was 16 to 48 percent of total body weight in males and 24 to 67 percent in females. These figures support our clinical impression that some of the subjects were obese.

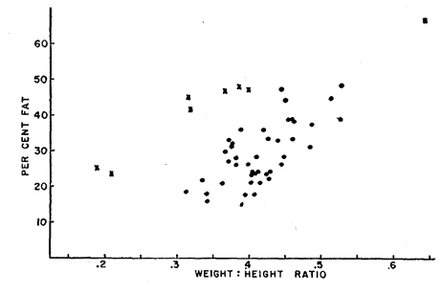

Fig. 2. Percentage of fat versus weight/height ratio (kg/cm). Females (x), males (•).

The relationships between fat content as determined by K^{40} measurement and two other parameters of fatness-leanness are depicted in Figs. 1 and 2. The correlation coefficient of fat content against average skin-fold thickness is 0.80 for the males, and that of fat content against weight/height ratio is 0.56 (males only). The data on females are too few to justify calculation of correlation coefficients for this group. However, the graphs suggest that the trends are similar to those of the males. It is of interest that females tend to have a higher fat content for a given weight/height ratio than do the males.

This report is presented as a new approach to the estimation of fat content in living man. The procedure has the obvious advantage of being nontraumatic and devoid of hazard to the subject. Work is now in progress in a further attempt to assess the validity and accuracy of this method (*11*).

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Effect of Deuterium Substitution in Sympathomimetic Amines on Adrenergic Responses

Abstract. It was discovered that replacement of the α-hydrogens of tyramine and tryptamine by deuterium produces a marked intensification of the blood pressure effects and nictitating membrane contraction normally produced by these amines. The results are interpreted on the basis of kinetic isotope effects at the level of monoamine oxidase and clearly establish the importance of this enzyme in the limitation of responses when tyramine and tryptamine are involved. The observed deuterium isotope effects with α,α-bisdeuterotyramine (α,α-D_2-tyramine) have been reproduced with only one of the optical isomers of monodeuterotyramine. This establishes that the enzyme displays a high degree of optical specificity. The use of *l*-bisdeuteronorepinephrine revealed that norepinephrine cannot be attacked by the enzyme at the effector cell level.

The role of monoamine oxidase (MO) in relation to adrenergic mechanisms has long been a matter of some controversy (*1*). It was suggested some time ago (*2*) that the excitatory properties of drugs such as amphetamine or ephedrine were at least partly related to their inhibitory properties towards monoamine oxidase. In more recent years, the discovery of potent new inhibitors has made it possible to establish an important role for this enzyme in adrenergic mechanisms (*3*). Thus, the administration of iproniazid could be shown (*4*) to potentiate the action of various adrenergic amines, such as tyramine, on the nictitating membrane of the cat. However, no potentiation of the action of the normal transmitter norepinephrine could be demonstrated (*4*). This would seem to indicate that termination of the action of norepinephrine and epinephrine does not involve breakdown through attack by monoamine oxidase. It has been shown by Axelrod and his collaborators that O-methylation is in all probability the mechanism responsible for inactivation of catecholamines (*5*) although, according to Brodie and his group, monoamine oxidase may be involved in the disposition of catecholamines in brain (*6*). However, the demonstration by Carlson (*7*) of the presence of appreciable concentrations of dopamine in brain (as well as serotonin) provides a possibility that the central effects of iproniazid could be related in some way to increased levels of dopamine and serotonin (*6*), both of which can act as substrates for monoamine oxidase.

It has not yet been possible to assess quantitatively and unambiguously the role of monoamine oxidase in adrenergic mechanisms because of the use of large doses of noncompetitive inhibitors