nucleus of the cat are depressed during attentive behavior (*1*). The authors attributed this effect to a reticular activating influence, based on the fact that they had observed a similar reduction of the dorsal cochlear nucleus potential in "encéphale isolé" cat, during reticular stimulation. It was therefore deduced that transmission in the first synapse of the acoustic pathway is inhibited by reticular discharges. This deduction provided the basis for an attractive theory, according to which reticular formation controls the first synapses of all sensory pathways (*2*).

However, our experiments have shown that injections of curare made in the "encéphale isolé" preparation suppress the reduction in amplitude of the dorsal cochlear potential induced by reticular stimulation. This suggests that tympanic muscles are responsible for the reduction of the response of the cochlear nucleus during arousal.

Reduction in amplitude of the microphonic potential recorded on the round window of the cochlea is the classical test of the contraction of middle ear muscles; since mesencephalic reticular stimulation induces this reduction (Fig. 1), it can be assumed that the reticular excitation controls sound transmission at the middle ear level by way of tympanic muscles. These muscles mechanically attenuate the pressure transmitted from the eardrum to the oval window through the ossicular chain and lessen the amplitude of acoustic stimuli reaching the cochlea.

After disinsertion of the tympanic muscles from the middle ear ossicles, reticular stimulation fails to induce any diminution of the cochlear response. Figure 2 shows potentials evoked simultaneously in the left and right cochlear nuclei. The responses recorded from the side which has intact middle ear muscles are reduced by reticular stimulation (lower beam) but are not modified on the side where stapedius and tensor tympani have been removed (upper beam). This implies that no inhibition of reticular origin takes place at the first relay of auditory pathways and that reduction in amplitude of cochlear nucleus potentials is a purely passive phenomenon.

From the point of view of motricity, the delay, evolution, and control of the contractions of tympanic muscles, under reticular stimulation, can be compared with the delay, evolution, and control of other motor facilitations induced by reticular activation. Furthermore, observation of the "encéphale isolé" cat's face during the reduction of the cochlear nucleus response by reticular excitation shows the pattern of muscular contraction such as has been described by Hess (*3*) as the facial component of the "tegmental motor reaction" of Thiele. Therefore the reticular control of auditory input may be understood to be the result of an infraliminal reflex facilitation, belonging to a generalized motor reaction (*4*).

Considered from the point of view of auditory sensation, contractions of tympanic muscles appear unimportant. Our experiments have shown that the diminution of microphonic potentials has never been greater than 13 db. Even in the case of reticular stimulation with powerful arousing action on the corticogram, the mean reduction registered was still under 5 db. This would explain that near-threshold responses only are reduced by reticular stimulation at the cochlear nucleus level. Therefore, in normal conditions of wakefulness, the reticular control of auditory input appears no more important than other aleatory modifications occurring through active behavior (masking effect, head orientation, and so forth) (*5*).

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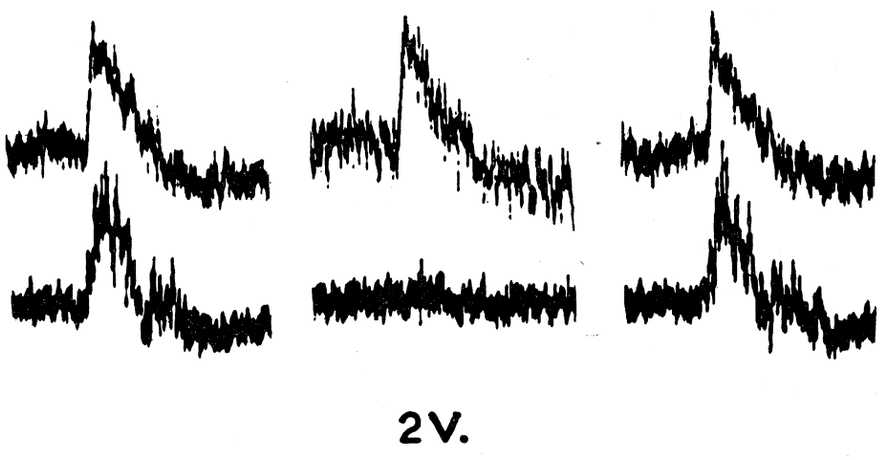

Fig. 2. Dorsal cochlear nucleus potential evoked by juxtaliminal clicks each second. Suppression of the attenuating reticular effect (2 volts, 300 cy/sec) on one side (upper beam) by cutting the tendons of tensor tympani and stapedius. Positivity upwards.

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Technique for the Study of Alternate Metabolic Pathways; Epinephrine Metabolism in Man

Abstract. A general method of determining the relative magnitudes of different pathways of formation of a urinary metabolite from a single precursor substance is described. The method requires the administration of the precursor and an intermediate labeled with different isotopes, and the determination of the ratio of the isotopes in the metabolites. A preliminary application to epinephrine metabolism in man is presented.

The metabolic conversion of one compound to another, through more than one pathway, presents a special problem with regard to ascertaining the relative magnitude of each of the pathways. When an intermediate compound is also a major excretion product, it is possible to state which is the major pathway (*1*). This, however, introduces unnecessary variances, since more than one experiment is required, and it is not possible to appraise the extent to which each pathway is used or to determine the proportion of a final metabolite formed via a given pathway.

The present report shows that this information can be obtained in vivo, under different conditions or disease states, in a single experiment, by administering, simultaneously, compounds appropriately labeled with different isotopes and determining the ratios of the isotopes found in each of the excreted metabolites. When the total radioactivity in each metabolite can also be determined, the magnitude of each of the pathways may be expressed in terms of a percentage of the precursor substance. The development of a convenient method for the simultane-

ous determination of H^3 and C^{14} (*2*) has overcome many of the technical difficulties, and the principles of the method here described should find wide application in the study of alternate metabolic pathways. As an example of the type of problem encountered, and its solution by the double labeling technique, the metabolism of epinephrine has been studied, by using epinephrine-7-H^3 and metanephrine-methoxy-C^{14}.

Epinephrine can be metabolized by both monoamine oxidase (*3*) and O-methyltransferase (*4*). Figure 1 summarizes the known pathways of metabolism of epinephrine in man. The metabolites that have been shown to be present in urine (*5*) as well as probable intermediates, are indicated. The rate constants of the reactions are designated by K_{XY}, where the subscripts refer to the precursor X and the product Y. The fraction of a metabolite entering any given reaction can be shown to be equal to the rate constant of that reaction divided by the sum of the rate constants of all the reactions (or transfers) through which the substance leaves its metabolic pool. The fraction of epinephrine converted to metanephrine is

$$f_{EM} = \frac{K_{EM}}{K_{EM} + K_{EU} + K_{EC} + K_{ED} + K_{EO}}$$

and the fraction of metanephrine conjugated is

$$f_{MC} = \frac{K_{MC}}{K_{MC} + K_{MU} + K_{ML}}$$

The product of these fractions is the portion of an administered dose of epinephrine that would be excreted as conjugated metanephrine. If epinephrine-7-H^3 were administered, the amount of tritium excreted as conjugated metanephrine-7-H^3 would be $f_{EM} \cdot f_{MC} \cdot H^3_0$ (where H^3_0 is the total tritium in the epinephrine given). Similarly, the proportion of C^{14}_0, administered as metanephrine-methoxy-C^{14}, excreted as the conjugate would be $f_{MC} \cdot C^{14}_0$ (where C^{14}_0 is the administered dose). The H^3/C^{14} ratio in conjugated metanephrine after all radioactivity has been excreted would therefore be:

$$\left[\frac{H^3}{C^{14}}\right]_{\substack{\text{conjugated}\\ \text{metanephrine}}} = f_{EM} \cdot \frac{H^3_0}{C^{14}_0} \qquad (1)$$

Since H^3_0/C^{14}_0 is known, the fraction of epinephrine metabolized to metanephrine can be calculated.

The total H^3 and C^{14} present in 3-methoxy-4-hydroxymandelic acid (VMA) and 3-methoxy-4-hydroxyphenylglycol (MHPG) and their H^3/C^{14} ratios can also be expressed in terms of these fractions:

$$\left[\frac{H^3}{C^{14}}\right]_{VMA} = \frac{f_{EM} f_{ML} f_{LV} + f_{ED} f_{DA} f_{AV}}{f_{ML} f_{LV}} \cdot \frac{H^3_0}{C^{14}_0} \qquad (2)$$

$$\left[\frac{H^3}{C^{14}}\right]_{MHPG} = \frac{f_{EM} f_{ML} f_{LG} + f_{ED} f_{DR} f_{RG}}{f_{ML} f_{LG}} \cdot \frac{H^3_0}{C^{14}_0} \qquad (3)$$

The portions of these substances formed from epinephrine via the pathway through metanephrine are:

$$\text{VMA} = \frac{f_{EM} f_{ML} f_{LV}}{f_{EM} f_{ML} f_{LV} + f_{ED} f_{DA} f_{AV}} \qquad (4)$$

$$\text{MHPG} = \frac{f_{EM} f_{ML} f_{LG}}{f_{EM} f_{ML} f_{LG} + f_{ED} f_{DR} f_{RG}} \qquad (5)$$

The fractions in Eqs. 4 and 5 may be calculated by dividing the left-hand member of Eq. 1 by the left-hand member of Eq. 2 and the left-hand member of Eq. 3, respectively. If the total H^3 appearing in VMA and MHPG is also known, the quantity of H^3 forming VMA and MHPG via metanephrine can be determined and expressed as a percentage of the injected H^3 epinephrine (H^3_0). Thus the relative magnitude of each pathway in the formation of VMA and MHPG can be determined and the fraction of epinephrine converted to metanephrine estimated.

Because the metanephrine-methoxy-C^{14} is injected into the blood stream, excessive amounts are excreted into the urine in the free form. If the injected metanephrine behaved exactly as endogenously produced metanephrine, the H^3/C^{14} ratios of free and conjugated metanephrine would be identical. If the ratios are not the same, the excess free metanephrine-C^{14} would result in a lower H^3/C^{14} ratio in this compound. This amount of C^{14} never really entered the metabolic pool and must be subtracted from the administered C^{14} in determining H^3_0/C^{14}_0.

The excess C^{14} in free metanephrine

Table 1. Urinary excretion of radioactive compounds during the 48 hours following the intravenous administration of 38.4 μc of L-epinephrine-7-H^3 and 4.58 μc of L-metanephrine-methoxy-C^{14}. Results are expressed in microcuries.

Compound isolated	Subject G. J.			Subject D. W.		
	H^3	C^{14}	H^3/C^{14}	H^3	C^{14}	H^3/C^{14}
Metanephrine						
Free	2.63	0.568	4.62	2.08	0.395	5.28
Conjugated	9.34	1.64	5.68	7.34	1.31	5.62
3-Methoxy-4-hydroxymandelic acid	17.0	1.34	12.7	17.3	1.45	11.9
3-Methoxy-4-hydroxyphenylglycol	2.12	0.189	11.2	2.43	0.204	11.9
Epinephrine (free and conjugated)	1.23			1.26		

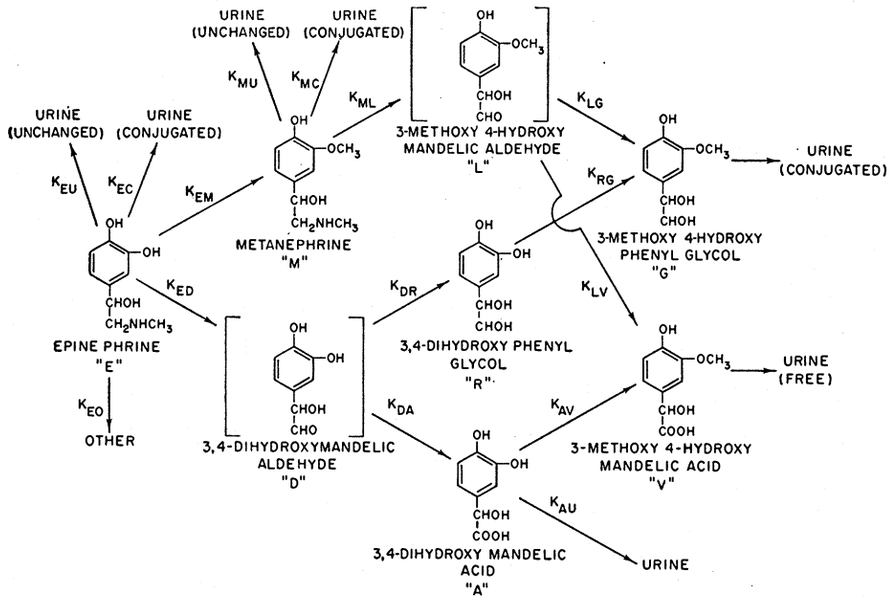

Fig. 1. Epinephrine metabolism in man. Compounds in parentheses have not been demonstrated. 3,4-Dihydroxyphenylglycol has been tentatively identified only in the urine of rats treated with pyrogallol.

can be estimated from the difference in ratios:

$$\left[C^{14}\right]_{\substack{\text{excess}\\ \text{in free}\\ \text{metanephrine}}} = \left(\left[\frac{C^{14}}{H^3}\right]_{\substack{\text{free}\\ \text{meta-}\\ \text{neph-}\\ \text{rine}}} - \left[\frac{C^{14}}{H^3}\right]_{\substack{\text{conju-}\\ \text{gated}\\ \text{meta-}\\ \text{neph-}\\ \text{rine}}}\right)\left[H^3\right]_{free}$$

The correction of the injected metanephrine C^{14}, by subtraction of the excess urinary free metanephrine-C^{14}, is necessitated by the initial rapid excretion of the injected compound. In order to be conjugated or metabolized, however, the injected metanephrine-C^{14} must enter the tissues. It is assumed that the lag in the initiation of these reactions is similar and that metanephrine and epinephrine can enter the various tissues with equal ease, so that a correction for conjugated metanephrine is unnecessary.

Using these principles, I made an attempt to evaluate the pathways of metabolism of epinephrine in man. Two normal males received 38.4 μc of L-epinephrine-7-H^3 and 4.58 μc of L-metanephrine-methoxy-C^{14} (*6*), and the various metabolites were isolated (*5*) from the urine collected during the following 48 hours. The amounts of H^3 and C^{14} in each compound were determined simultaneously in a liquid scintillation counter (*2*). Table 1 indicates the distribution of radioactivity in the various compounds isolated from the urine. From the ratios of H^3/C^{14} found for each compound in the equations outlined, it was calculated that 66.1 percent and 68.0 percent of the injected epinephrine was methylated to form metanephrine. Of this, 20 percent and 21.4 percent formed VMA and 2.25 percent and 3 percent formed MHPG. Since 44.4 percent and 45.2 percent of the injected epinephrine formed VMA, 24.4 percent and 23.8 percent must have been formed by deamination followed by methylation. Similarly, 2.25 percent and 3.3 percent of the MHPG was formed by deamination followed by methylation. A total of 95.8 percent and 98.1 percent of the injected epinephrine could be accounted for by methylation to metanephrine, deamination prior to methylation to form VMA and MHPG, and excretion as unchanged or conjugated epinephrine.

The use of simultaneous labeling of two different metabolites in determining the relative importance of alternate pathways of metabolism can be widely applied (*7*).

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7. I would like to express my appreciation to Dr. Seymour S. Kety for his interest and encouragement, as well as his suggestions, in the development of this method.

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On the Thermal Boundary Layer of the Ocean

Abstract. Measurement of the long-wave infrared radiation from the top 0.1 mm of the evaporating ocean demonstrates the existence of a cool surface layer characterized by departures of as much as 0.6°C from the "surface temperature" found by conventional methods. Being very thin, the layer cools sufficiently rapidly to reestablish itself in less than 12 seconds after disruption by a breaking wave.

By means of simultaneous measurements of the radiation temperature and of the conventional thermometric temperature of the ocean, we have found evidence of a persistent cool boundary layer. The equipment used, shown in Fig. 1*A*, consisted of a double-beam radiometer having a spectral sensitivity in the band from 6 to 20 μ, a region in which the absorption in water is so high that 98 percent of the radiant flux originates in the first 0.1 mm. To minimize the necessary corrections for absorption by air and for reflection, the measurements were made at normal incidence at night from a position 2 m above the water. The water was shaded as required. For comparison, the temperature of the water beneath the radiation layer was measured by thermistors encapsuled in 1-mm glass beads, at depths dictated by the surface conditions of swell, waves, and ripples. The system had over-all sensitivity sufficient to discriminate temperatures with an uncertainty of less than 0.1°C, with response of less than 1 second.

Ocean measurements were made from the Scripps pier at a point 200 m off shore in water 7 m deep. A sample traced from the data is shown in Fig. 1*B*, which includes, for comparison, the "surface temperature" obtained by thermistor. The latter was checked by bucket sampling and mercury thermometer. The radiation temperature, when the ocean was shaded from the clear, cold night sky, was more than 0.7°C lower than the thermistor temperature. Since the screen used was at air temperature and therefore cooler than the ocean, a correction of +0.1°C must be applied to the measured value, which leaves a departure of the radiation temperature of more than −0.6°C. It should be noted that the conditions of wind and humidity were not conducive to vigorous evaporation. The effect of exposing the ocean to the night sky by removing the shade is readily seen in the right-hand section of the figure.

In order to evaluate the effect of breaking waves, a small electric rotary pump was submerged beneath the radiometer, positioned so as to draw water from a depth of 15 cm and direct it as a jet which welled up in the radiometer's field of view. It was determined by bathythermogram and thermistor that the water below the upper centimeter was isothermal within measurable limits. The result of intermittent pumping is shown in Fig. 1*C*. When the pump was run sufficiently vigorously to rupture the surface in the manner of a bubbling spring, the radiation temperature rose to approximately the values measured by the thermistor submerged at the level of the pump intake. When the pump was shut off, the radiation temperature dropped to its normal value in about 5 seconds, the cooling rate indicating that the effect takes place in a layer less than 1 mm thick. A remarkable finding was that less intense disturbance of the water failed to produce measurable effects.

Radiometric measurement over a breaking wave gave a concordant result. Coincident with the breaking, a momentary small warm signal was recorded, followed by a longer-lasting, stronger cold signal which seemed to coincide with the life span of the blanket of foam left behind by the wave. The whole disturbance lasted about 12 seconds; then the radiation returned to its normal value. Thus, on the open sea where whitecaps occur at a given point at relatively long intervals, the thermal boundary layer should be present, at least intermittently.

The chief features observed on the ocean were modeled in a controlled laboratory environment. In Fig 1*D* is shown, at the left, the radiation temperature of a salt-water surface being gradually warmed by radiation from the ceiling and walls of the room. Under these conditions, the heat flux was downward, and the water was initially in a state of stable stratification with a warm surface layer. As indicated in the figure, a fan was caused to blow periodically on the surface, the air stream having a velocity of about 1 m/sec and a relative