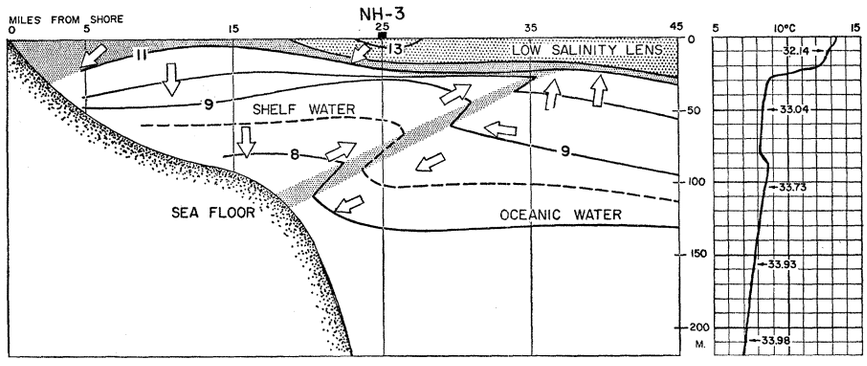

Fig. 1. (Left) Temperature-depth section off Newport, Oregon, during October 1960. (Right) A copy of the bathythermogram recorded in the center of the section, at the line marked NH-3. The salinity is given for the depths where it was measured.

haps this front (*3*) was real and constituted a boundary between oceanic water and shelf water that had been modified during and after summer upwelling.

Figure 1 illustrates the proposed analysis and shows (at right) a bathythermogram recorded in the center of the temperature-depth section, at station NH-3. The vertical lines in the section represent the sites of the observations; casts were to 200 m or to the bottom, whichever distance was less. The depths of the isotherms were determined from the various traces. When a positive gradient appeared, the positions and temperatures for the minimum and maximum were read. The arrows indicate changes that had occurred in the positions of the isotherms since the previous month. The lightly shaded water between the 11-degree isotherm and the surface is a local low-salinity water, separated by a sharp thermocline and halocline from the waters lying beneath it (the pycnocline is shaded more deeply). The sloping shaded zone represents the front.

Figure 1 shows conditions in October 1960; observations were made about once a month throughout the year and are continuing (*4*). No inversions appeared in the data for August; in September, inversions appeared in positions close to those shown in Fig. 1, and a frontal analysis similar to the one shown could be made. Between September and October the water below the thermocline, but above the front, cooled a few tenths of a degree centigrade. For a few meters below the front the water warmed a few tenths of a degree. At the two inshore stations the water all the way to the bottom was warmer in October than in September. Offshore, there was cooling at the surface but relatively little change below the thermocline. By late November, inversions appeared on the two offshore observations only, instead of on the two central ones, and inversions appeared again on the two offshore observations in January, but at somewhat deeper levels. No cruise was made during February; in March and April no inversions were found along this line.

The surface circulation in this region is wind-driven. Longshore currents to the south during the summer are accompanied by vigorous upwelling; alongshore water that is normally found at depths of 100 to 200 m appears at the surface. Usually in August this upwelling lessens, and occasional currents to the north are observed. During the fall and winter, northward-flowing currents are the rule. As these currents change, the distribution of mass near shore adjusts. As the upwelling lessens, warmer surface water moves inshore, and the water that upwelled previously runs down off the shelf to its more normal location. If, however, it has been modified at the surface by warming during the summer, and especially by mixing with low-salinity surface waters and local runoff, it will have characteristics different from those of the oceanic waters at the levels to which it returns. Under some circumstances it may override the saltier oceanic mass, as suggested in Fig. 1.

Data were also collected off Astoria and Coos Bay, about 100 miles north and south of Newport, respectively. Although occasional positive gradients appeared, they did not seem to indicate a continuous frontal surface, and there was no special coherence in pattern from one month to the next. The bottom slopes more steeply at each of these locations than it does off Newport, where there are shoals offshore. Perhaps these shoal areas played a significant part in maintaining the pattern observed in the Newport section.

This is not the only way in which isotherms can be drawn, of course, and the type of analysis may lead to some of the flow pattern suggested by the arrows. Furthermore, since positive gradients are frequent in the area, one cannot overlook the possibility that these were simply introduced either from the north or the south, or from offshore. Nevertheless, the analysis is consistent with all of the data, and the mechanism appears to be possible. (*5*).

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3. The term *front* was used by T. Cromwell and J. L. Reid, Jr., [*Tellus* **8**, 94 (1956)] to describe a convergence zone delineated by a sharp decrease in temperature with depth, if that zone appeared along some line at the surface.
4. We are indebted to Bruce Wyatt of Oregon State University, who made the observations mentioned, for drawing our attention to the bathythermograms on which this report is based.
5. This work was supported by the Office of Naval Research.

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Uptake of Catecholamines by a Particulate Fraction of the Adrenal Medulla

Abstract. Chromaffin granules isolated from beef adrenal medulla took up C^{14}-labeled catecholamines from an isotonic medium at 37°C at a rate approximately 20 times the rate at 0°C. The uptake was stimulated three- to five-fold by Mg^{++} and adenosine triphosphate. Reserpine ($1 \times 10^{-5} M$) caused a 90 percent inhibition of uptake; ethylenediaminetetraacetic acid completely inhibited the stimulation by magnesium and adenosine triphosphate, and the inhibition could be reversed by addition of excess Mg^{++}.

The occurrence of catecholamines and adenosine nucleotides in the chromaffin granules of the adrenal medulla (in an approximate molar ratio of 4:1) has suggested that these compounds are associated in a non-diffusible complex within the granules (*1*). Previously Hillarp (*2*) reported that only small amounts of tritiated adrenaline were taken up by chromaffin granules at 0°C and attributed this small uptake to lack

of exchange between intragranular and external amine. The experiments reported here demonstrate that the chromaffin granules remove C^{14}-labeled catechlolamaines from the surrounding fluid at 37°C by a process which is stimulated by adenosine triphosphate (ATP) and inhibited by reserpine.

Chromaffin granules were isolated by the procedure described by Hillarp (*3*). After the second washing the granules were diluted with 0.3*M* sucrose to give a suspension equivalent to 0.5 to 1.0 g of medulla per milliter. The weight of the granules was determined by weighing a pellet obtained by centrifuging a portion of the suspension at 26,000*g* for 20 minutes. The granules were incubated at 37°C in 3 ml of an isotonic medium containing either C^{14}-labeled adrenaline, noradrenaline, or hydroxytyramine, and then they were transferred to cold centrifuge tubes and chilled to 0°C. The reaction vessels were rinsed with 2 ml of cold 0.3*M* sucrose which was added to the granule suspension. The granules were then sedimented by centrifuging at 26,000*g* for 10 minutes at 5°C. The pellet was washed twice by suspending it in 5 ml of cold 0.3*M* sucrose and allowing the mixture to stand in ice for 30 minutes. After the second wash, the granules were lysed by adding 5 ml of ice-cold distilled water. Samples of the incubation medium, of each of the washes, and of the lysate were assayed for radioactivity in a Packard Tri-Carb liquid scintillation spectrometer. When adrenaline or noradrenaline was used, paper chromatography of the radioactivity in the lysate showed that it contained the original, unchanged compound. When hydroxytyramine-C^{14} was incubated with the granules in an atmosphere of 95 percent oxygen and 5 percent carbon dioxide, about 40 percent of the total radioactivity in the lysate was due to noradrenaline; analysis of the incubation medium indicated only slight amounts of noradrenaline, essentially all of the radioactivity being due to hydroxytyramine. Thus, practically all of the newly formed noradrenaline was retained within the granules.

Table 1 shows the effect of increasing concentrations of ATP on the incorporation of adrenaline-C^{14} by the chromaffin granules. Addition of 10 μ mole of ATP to the medium produced a two- to threefold stimulation of uptake; addition of both Mg^{++} and ATP produced a three- to fivefold increase while Mg^{++} by itself had no effect. In the presence of ATP and Mg^{++} the rate of uptake of the catecholamines at 37°C was about 20 times as great as the rate at 0°C.

Table 1. Effect of ATP on the uptake of adrenaline-C^{14}. Each vessel contained 5 μmole of $MgCl_2$, 150 μmole of phosphate buffer (*p*H 7.0), 1 mg of iproniazid phosphate, 66 mg (wet weight) of chromaffin granules, and 0.1 μmole of *dl*-adrenaline-C^{14} (specific activity 1 μc/μmole, or 8.5×10^5 count/min) in a total volume of 3 ml of 0.3*M* sucrose. Incubated at 37° for 30 minutes in air. The data in parts A and B were obtained with two different granule preparations.

ATP added (μmole)	Radioactivity (10^3 count/min)			
	Incubation supernatant	1st wash	2nd wash	Granule lysate
		Part A		
0	82.1		0.6	3.8
2	72.3	2.4	0.8	9.7
5	65.0	2.6	0.8	14.4
10	63.0	2.4	1.1	17.8
15	65.8	2.2		19.3
20	64.4	2.4	1.6	18.7
		Part B		
10	58.2	4.1	3.1	28.4
10*	94.8	1.0	0.2	1.7

*Contained reserpine ($10^{-5}M$).

Table 1 also shows the inhibition of uptake caused by reserpine. Maximum inhibition was produced by $10^{-5}M$ reserpine; $10^{-7}M$ reserpine caused 50-percent inhibition. The inhibition caused by reserpine was not reversed by washing the granules in 0.3*M* sucrose and dialysis overnight. Ethylenediaminetetraacetic acid (EDTA) also inhibited the uptake of catecholamines, but its inhibition differed from that of reserpine in two ways. First, EDTA completely inhibited the stimulation of uptake caused by addition of Mg and ATP but had little effect on the uptake in the absence of Mg and ATP. Second, the EDTA inhibition was completely reversed by addition of excess Mg^{++}.

The fact that reserpine also inhibited the uptake of hydroxytyramine by chromaffin granules may offer an explanation of the depletion of catecholamines in the adrenal medulla and other tissues caused by reserpine in vivo. When chromaffin granules were incubated with hydroxytyramine-C^{14} in the presence of $10^{-5}M$ reserpine, uptake was inhibited by 83 percent. Chromatographic analysis of the granule lysate and of the incubation medium after removal of the granules showed only a trace of noradrenaline in the granule lysate and no detectable noradrenaline in the incubation medium. In the absence of reserpine, 25 percent of the added hydroxytyramine was taken up by the granules, of which 34 percent was converted to noradrenaline and remained within the granules. Since hydroxytyramine is formed in the cytoplasmic sap and converted to noradrenaline only after it is taken up by the chromaffin granules, inhibition of hydroxytyramine uptake would prevent synthesis and replenishment of the catecholamines lost through normal physiological processes.

Since the chromaffin granules contain large amounts of ATP and since it has been postulated that ATP is involved in the storage of catecholamines, it was of interest to determine whether granules could remove ATP or adenosine diphosphate (ADP) from the medium and, if so, whether such uptake is affected by addition of adrenaline or noradrenaline. When the granules were incubated under conditions in which they incorporated C^{14}-labeled adrenaline or noradrenaline, no incorporation of labeled ATP or ADP was observed. Chromatography of the incubation medium showed that most of the ADP and ATP was hydrolysed to adenosine monophosphate (AMP); thus external AMP is likewise not taken up by the chromaffin granules. Incubation of the granules with P^{32}-labeled inorganic phosphate, inorganic pyrophosphate, or ATP revealed that no P^{32} from any of these compounds was incorporated into intragranular ATP under conditions in which adrenaline was taken up.

The stimulation of uptake by Mg and ATP, the differences in the inhibition caused by reserpine and EDTA, and the 20-fold increase caused by a 37°C change in temperature indicate that the uptake is more dependent on energy than diffusion and intragranular binding. The fact that, immediately upon lysis of the granules, the catecholamines are freely diffusible and do not appear to be associated with any other molecular species indicates that, if they are held in some complex within the granule, it is only by very weak binding forces (*2; 4*).

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