of oxygen. The supernatant, now being in B, is drawn off through the vacuum line after opening stopcock B. After flushing B and D with several changes of buffer, buffer is drawn into tube A and B, nitrogen is admitted to equalize the pressure, stopcock C is closed, and the centrifuge unit is removed. After shaking gently to stir up the sediment, the preparation is centrifuged and washed the required number of times as described previously. The centrifuge unit has been spun at 2200 rpm without breaking. Under these conditions the average relative centrifugal force was 900, and the maximum at the bottom of the tube was about 1500 times gravity.

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The Inhibitory Action of Hydroxylamine on Biological Oxidations

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Hydroxylamine is used as a reagent for acylphosphates (*1*) and acylmercaptans (*2*) and, in the concentration of 0.02 *M*, as a detecting agent for these compounds in enzymatic systems (*3*).

The oxidation of various substrates by rat liver mitochondria, isolated in isotonic sucrose, is strongly inhibited by 0.02 *M* of hydroxylamine.

TABLE 1

INHIBITION OF OXIDATION*

Expt	Substrate	O_2 uptake: Without	O_2 uptake: With NH_2OH	Time min	Inhibition %
1	*l*-Malic	99.5	50.3	30	49.5
2	Fumaric	31.5	12.6	15	60.0
3	Succinic	62.5	49.5	20	20.8
4	*l*-Glutamic	54.0	14.2	22	73.6
5	Succinic†	107.5	77.1	20	28.7
6	*l*-Glutamic‡	105.0	4.0	22	96.2

* Frozen Warburg vessels contained $MgSO_4$ 0.05 *M*, NaF 0.005 *M*, glycylglycine 0.02 *M*, adenosine-5-phosphate 0.004 *M*, orthophosphate 0.006 *M* pH 7.4, substrate 0.025 *M*, $NH_2 \cdot OH$ 0.02 *M*. Final pH 7.4. At 0 time 0.5 ml of mitochondria suspension in isotonic KCl. Gas phase air, temperature 20–25° C. Results are means of three or more flasks. Different substrates used with different mitochondria preparations.

† Enzyme aged for 15 min at 37° C.

‡ With 2,4-dinitrophenol 2.10^{-4} *M*.

Expts. 5 and 6 show that the inhibition remains even when oxidative phosphorylation is uncoupled by 2,4-dinitrophenol (DNP) or by aging at 37° C.

The inhibition is, at least, partly due to the combination with iron enzymes (*4*). In our conditions 0.02 *M* of hydroxylamine inhibited 100% the oxidation of added reduced cytochrome c, to mitochondria preparations.

Another point of inhibition exists, and it is shown by inhibition of the reduction of methylene blue in Thunberg tubes.

TABLE 2

INHIBITION OF DEHYDROGENASE SYSTEMS*

Substrate	Other conditions	ΔD, 660 mμ	$NH_2 \cdot OH$
l-Malic	—	34	4
Succinic	—	44	5
l-Glutamic	2.10^{-4} *M* DNP	44	2
l-Glutamic	15.10^{-3} *M* arsenate	36	2
l-Glutamic	Previous incubation with 1 mg riboflavin	47	3
Choline chloride†		90	5

* Thunberg tubes contain 2 ml orthophosphate buffer 0.125 *M* pH 7.4; 0.2 ml methylene blue 1/5000; 0.3 ml mitochondria suspension. In the sidearm 0.4 ml substrate 0.4 *M*. After evacuation and tipping, reaction was followed by direct reading at 660 mμ in Klett photocolorimeter.

† K_2HPO_4 0.01 *M* extract of guinea pig liver, instead of mitochondria.

No inhibition was found with crystalline alcohol dehydrogenase from yeast (*5*), nor with crude glutamic dehydrogenase from rat liver (*6*) measured by spectrophotometric determination of DPN reduction.

Diaphorase was prepared by the method of Edelhoch *et al.* (*7*), and its activity was measured in Thunberg tubes with methylene blue, diphosphopyridine nucleotide (DPN), and alcohol dehydrogenase. 2,6-Dichlorophenolindophenol cannot be used because it is reduced by hydroxylamine. Neotetrazolium confirms the results obtained by methylene blue, and it is not directly reduced by dehydrogenase-DPN, in absence of diaphorase. Hydroxylamine, in the same concentration, inhibited 90–100% diaphorase preparation before the alcohol step, but not after. The inhibition can be restored by the addition of alcohol insoluble protein. Trichloroacetic filtrate or boiled extract does not have this activity.

TABLE 3

INHIBITION OF DIAPHORASE*

Enzyme	Add	Direct reading 660 mμ: $NH_2 \cdot OH$ 0.02 *M*	Direct reading 660 mμ: —
Crude	—	9	79
Alcohol purified	—	86	85
"	0.2 ml alcohol insol. protein	8	84
"	Same & trichloroacetic filtrate	86	85
"	Same boiled 2 min	89	82

* In Thunberg tubes, 2 ml orthophosphate buffer 0.125 *M* pH 7.4; 0.1 ml alcohol dehydrogenase (100 units); 0.2 ml methylene blue 1/5000 0.1 ml diaphorase. 0.1 ml alcohol in sidearm. After evacuation and tipping reaction was followed by direct reading at 660 mμ in Klett photocolorimeter.

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Cytochrome c reductase activity of the same preparations is not inhibited by hydroxylamine. Hydroxylamine does not reduce reduced cytochrome c. These inhibitions must be kept in mind when using hydroxylamine as a trapping reagent.

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Intragastric Temperature Variations in Man During Hunger

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The temperature of an area of the body or an organ must be looked upon as variable. Regional temperature is dependent upon rate of blood flow, oxygen consumption, and heat gained or lost through outside influences. The emotional state of the individual, by causing sweating, blushing, or blanching, has a direct bearing on regional temperature, as the local vascular response is regulated by nervous impulses.

Tissue respiration is directly dependent upon blood flow. It is, therefore, important to think of the body architecture as physiological anatomy. In the light of rational and conservative body response, the blood requirements of an organ or tissue at rest would not be equal to the requirements of the same structure during the different degrees of activity. It becomes obvious, therefore, that shunting or bypassing should be a normal physiological phenomenon. Blood is diverted to the tissue or organ with the greatest need.

Since a change in temperature follows upon any regional change in blood flow, and therefore oxygen exchange, experiments were devised to demonstrate the relationship between the vascular pattern and intragastric temperatures. It seemed reasonable to suppose, therefore, that, if the blood volume and rate of flow to an area were increased, the temperature would rise; and that consequently, if the blood volume and rate of flow were reduced, the temperature in the area would fall.

For this study we made a thermopile of iron constantan wires threaded through a gum rubber tubing. The tips of the individual thermocouples were insulated with *sauereisen* and then covered with a rubber plastic. This thermopile was made up of eight thermocouples, separately insulated. The thermopile was conducted to a fixed cold junction in a Thermos bottle. Although these elements were insulated, they were immersed in an ice bath of distilled water. The Thermos bottle was grounded and the thermopile was connected by two leads, well insulated, to a specially constructed potentiometer with a 10-deg range and a 24-hr automatic recorder. The thermopile was then passed through the mouth into the stomach with little effort from the patient.

The position of the tip of the thermopile was checked by fluoroscopy. With the tube in place, the leads were attached to the potentiometer. The temperature of the cold junction was checked, and the potentiometer was activated. The moment the switch was released, the inked stylus automatically recorded on a circular rotating chart. The oral temperatures were taken periodically and charted (Table 1).

TABLE 1

Subject	Oral temperature (°F)	Initial intragastric temperature (°F)	Hunger-phase intragastric temperature (°F)
D.B.	98.6	101.0	98.8
R.H.	98.8	100.6	99.4
K.H.	98.6	99.8	98.8
J.W.	97.8	100.0	99.2
S.K.	98.6	100.6	99.2
R.B.	98.2	100.7	100.2
D.C.	98.0	101.0	99.3
B.M.	97.8	100.0	99.0

Our subjects were male and female first-year medical students. They were asked to register immediately prodromal symptoms of hunger and restlessness, and they were by and large quite cooperative.

As noted in Table 1, the oral temperatures consistently ran 1–3 deg lower than the intragastric temperatures. In our experiments it was noted that approximately ½ hr after the patient began to desire food, the temperature took a sharp dip to a lower level; shortly after the subject's "hunger pangs" had subsided, the temperature within the stomach returned to its previous plateau or near it.

It was postulated that this reaction took place because the muscular contractions of the stomach arrested the blood flow and led to a dearth of oxygen and a reduction of regional metabolism. This contention is based on the previously published work done at this institution on the neurovascular mechanism of the stomach (*1, 2*). It has been demonstrated that in the stimulated stomach the mucous membrane is kept in a state of anemia because of the shunting mechanism of the vascular bed.

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