4. J. D. H. Strickland and T. R. Parsons, *Bull. Fisheries Res. Board Can. No. 125* (1960).
5. Since this report was written, A. DeVries and G. Kooyman of Stanford University have notified us of the collection of *Trematomus* sp. at the Koettlitz Glacier site. They also report the water at this site to have been "soupy with plankton" in late January 1962.
6. We thank members of U.S. Navy Task Force 43, of U.S. Naval Air Development Squadron Six, and of the U.S. Antarctic Research Program for their cooperation. This project was supported by National Science Foundation grant No. G-13209.

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Catalytic Dehydrogenation of Coal

Abstract. Coals were refluxed with palladium, ruthenium, and rhodium catalysts in high-boiling aromatic and heteroaromatic solvents. As much as 53 percent of the hydrogen in one coal was evolved as hydrogen gas. The yield of hydrogen varied with the rank of the coal and the nature of the solvent but was little influenced by the metal or catalyst support.

We report the first production of significant amounts of molecular hydrogen from coal by catalytic dehydrogenation. The results should help to elucidate the chemical structure of coal; and, since the amount of hydrogen obtained is surprisingly large, may lead to new uses for coal.

Several attempts have recently been made to estimate the hydroaromaticity of coal by dehydrogenation with sulfur (*1*) or iodine (*2*). The former method is open to question because of the complexity of the reactions taking place; indeed, it has been shown (*3*) that fully aromatic hydrocarbons are dehydrogenated by sulfur. The latter method is also uncertain, since little is known about the iodine dehydrogenation of model compounds.

We give here our results on the dehydrogenation of some vitrains (and related materials). A noble metal (palladium on calcium carbonate) was used as the dehydrogenating agent and a polycyclic aromatic compound such as phenanthridine was used as the solvent. This system offers the advantages that the hydroaromatic hydrogen is evolved as hydrogen gas, rather than as hydrogen sulfide or hydrogen iodide, and that the residual dehydrogenated vitrain is not contaminated, it being possible to remove the phenanthridine, calcium carbonate, and some of the palladium by treatment with hydrochloric acid. Phenanthridine was chosen because at its boiling point catalytic dehydrogenation usually proceeds at a rapid rate, and because it incorporates pyridine and phenanthrene structures, both of which are good solvents for coal. The conditions chosen were such as to give aromatization of hydroaromatic compounds, with few side reactions (see below). A mixture of freshly ground −200 mesh coal (0.50 g), catalyst (0.55 g), and phenanthridine (7.50 g) was refluxed with stirring for 5 hours in a helium atmosphere. For most coals, the bulk of the gas was evolved during the first hour. The gases (largely hydrogen, with small amounts of carbon monoxide, carbon dioxide, and methane) were analyzed by mass spectrometry, and the hydrogen was calculated as percentage of the total hydrogen in the coal which was evolved as hydrogen gas. Appropriate blank corrections were made. The results are summarized in Table 1. The amount of hydrogen evolved varies with the rank of the coal, from 15 percent for lignite vitrain to a maximum of 30 percent for Pittsburgh seam (Bruceton mine) vitrain, and then to 12 percent for Pocahontas vitrain. Kerogen evolved as much gas during the fifth hour as during the first; longer reaction time would thus give a higher yield of gas. Gilsonite behaved similarly.

The data in Table 2 show that as solvents, 5,6-benzoquinoline and 7,8-benzoquinoline are somewhat less effective than phenanthridine (3,4-benzoquinoline); that phenanthrene yields less hydrogen than phenanthridine, 5,6-benzoquinoline, or 7,8-benzoquinoline; that platinum group metals other than palladium, and supports other than calcium carbonate, give similar results; and that a commercial chromia-on-alumina dehydrogenation catalyst is ineffective. The use of 2-azafluoranthene as solvent gives the largest yield of hydrogen (53 percent, or 9400 cubic feet of hydrogen per ton of coal) yet obtained from Bruceton vitrain; it is not known whether this increased yield is caused by the increased reaction temperature or by some specific effect of the solvent. The dehydrogenated vitrains are hard and brittle, which may be evidence that condensation and cross-linking reactions have taken place.

When dehydrogenated with phenanthridine and palladium on calcium carbonate, 9,10-dihydroanthracene and 1,2,6,7-tetrahydropyrene give somewhat less than the amount of hydrogen theoretically required for complete aromatization; acenaphthene and fluorene yield 0.32 and 0.27 mole of hydrogen per mole of hydrocarbon; and anthracene, pyrene, and 3-methylphenanthrene give no hydrogen. With 2-

Table 1. Dehydrogenation of vitrains and related materials by 30 percent palladium on calcium carbonate catalyst in the presence of phenanthridine at 347°C.

Substrate	Element in substrate (moisture- and ash-free) (%)		Percentage of total hydrogen in sample evolved as H_2	Atoms of hydrogen removed per 100 carbon atoms
	Carbon	Hydrogen		
Vitrain, lignite, N.D.	67.9	5.5	14.6	14
Vitrain, Rock Springs, Wyo.	76.9	5.8	21.4	19
Vitrain, Pittsburgh, Pa.	83.3	5.4	29.7	23
Vitrain, Powellton, W. Va.	84.9	5.1	19.0	14
Cannel Coal, W. Va.	85.1	6.9	26.7	26
Vitrain, Pocahontas No. 3, W. Va.	90.1	4.6	12.1	8
Gilsonite (asphaltite), Eureka Mine, Utah	85.1	10.2	13.5	19
Kerogen (from Green River oil shale)	79.7	11.0	16.6	27

Table 2. Dehydrogenation of Pittsburgh vitrain with various catalysts and solvents.

Catalyst	Solvent*	Percentage of total hydrogen in sample evolved as H_2	Atoms of hydrogen removed per 100 carbon atoms
30% Pd-$CaCO_3$	2-Azafluoranthene	52.3	40
30% Pd-$CaCO_3$	2-Azafluoranthene†	53.1	41
30% Pd-$CaCO_3$	5, 6-Benzoquinoline	28.3	22
30% Pd-$CaCO_3$	7, 8-Benzoquinoline	20.4	16
30% Pd-$CaCO_3$	Phenanthrene	18.3	14
5% Pd-$CaCO_3$	Phenanthridine	32.0	25
1% Pd-$CaCO_3$	Phenanthridine	33.9	26
5% Pd-Al_2O_3	Phenanthridine	29.3	23
5% Ru-Al_2O_3	Phenanthridine	28.1	22
5% Rh-Al_2O_3	Phenanthridine	30.4	23
Cr_2O_3-Al_2O_3	Phenanthridine	0	0

* Reaction temperatures: 2-azafluoranthene, 384°C; 5,6-benzoquinoline, 349°; 7,8-benzoquinoline, 339°; phenanthrene, 333°. † Solvent : vitrain ratio 25 : 1 instead of the usual 15 : 1.

azafluoranthene and palladium on calcium carbonate, anthracene gives no hydrogen; fluorene gives 0.64 mole of hydrogen per mole of hydrocarbon.

Bruceton vitrain has thus been found to evolve about one-third of its hydrogen when dehydrogenated in the presence of phenanthridine, and about one-half when dehydrogenated in 2-azafluoranthene. Some of the hydrogen evolved with 2-azafluoranthene is probably due to side reactions; nevertheless, we believe that the hydroaromatic hydrogen in Bruceton vitrain is at least 30 percent, and may be considerably higher.

RAPHAEL RAYMOND
IRVING WENDER
LESLIE REGGEL
Pittsburgh Coal Research Center,
U.S. Bureau of Mines,
Pittsburgh 13, Pennsylvania

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Physiology of Acclimation to Low Temperature in Poikilotherms

Abstract. Potassium, sodium, and calcium increase and chloride, magnesium, and free amino acids decrease in cold acclimated fresh-water mussels and earthworms. Increased protein synthesis occurs. Neurosecretory cells of cold earthworms exhibit increased activity. Addition of cold worm body fluid stimulates increased O_2 consumption by normal tissues, indicating that a hormonal agent triggers the sequence of changes.

Since the pioneering studies of Fox (*1*), considerable evidence has accumulated which shows that many poikilotherms are able to compensate for temperature in their metabolism and activity (*2*). The degree of compensation is different in different groups of animals (*2, 3*) and the mechanisms underlying this compensation are not fully understood. It has been shown that the quantity of bound water changes (*4*). Several studies indicate that enzyme activity changes (*4, 5*). Changes in metabolic pathways have even been suggested (*5*). Pampapathi Rao and Ramachandra (*6*) reported recently that acclimation to high temperature results in changes in the level of free amino acids in the blood and body fluids of some invertebrates. We have, therefore, undertaken to find out the causes and consequences of the change in amino acid level in the hope of understanding the mechanisms underlying temperature acclimation. The present paper is a preliminary report of the results obtained from our study of cold acclimation in two tropical poikilotherms.

Table 1. Changes in the concentration of free amino acids and certain inorganic ions in the blood of the fresh-water mussel and the body fluids of the earthworm, after acclimation to low temperature. In all cases the data are based on an analysis of not less than 18 individuals. All figures are in millimoles per liter, except for the concentration of free amino acid, which is expressed as mg/100 ml.

State of acclimation	Free amino acids	Cl	Mg	K	Na	Ca
		Mussel, Lamellidens marginalis				
Normal 29°C	4.03 ± 0.5	14.7 ± 1.6	—	0.62 ± 0.04	18.8 ± 1.2	3.37 ± 0.37
Cold 19°C	2.3 ± 0.3	10.2 ± 1.0	—	0.73 ± 0.05	22.5 ± 1.4	5.63 ± 0.62
		Earthworm, Lampito mauritii				
Normal 29°C	104 ± 28	36.7 ± 4	8.9 ± 1	11.9 ± 3	38.5 ± 8	9.16 ± 1.2
Cold 19°C	56.2 ± 20	31.7 ± 4	6.5 ± 1.3	17.5 ± 3.5	49.8 ± 9	12.6 ± 2

The freshwater mussel, *Lamellidens marginalis,* and the earthworm *Lampito mauritii* were used in this investigation. The normal (room) temperature during these studies was 29° ± 1°C. In addition to animals kept at room temperature, several batches of individuals of both species were acclimated to 19° ± 1°C for 20 to 30 days in the laboratory. Blood of mussels (drawn directly from the heart) and body fluids of earthworms were analyzed for chloride, sodium, potassium, calcium, magnesium, and free amino acids in the normal as well as cold acclimated animals. There was an increase in sodium and potassium during cold acclimation in both species, while chloride, magnesium, and free amino acids decreased during cold acclimation. Calcium increased markedly in cold mussels but in cold earthworms the increase was not as great. Because potassium activates muscle metabolism (*7*), an increase in potassium in body fluids of cold acclimated forms will tend to increase muscle metabolism and this effect will be augmented when the calcium concentration also increases. These effects are further enhanced by the simultaneous decrease in magnesium (see Table 1).

An interesting result is the decrease in free amino acids in cold acclimated individuals. It was thought that this decrease might result from increased incorporation of amino acids into proteins of the cells, due to increased protein synthesis during cold acclimation. Individual blood cells in cold acclimated mussels were measured under the interference microscope (*8*) for total protein content (dry matter) and compared to those of normal individuals. There was a 21 percent increase in the protein content of the cells of cold acclimated individuals.

Nucleic acid content of different tissues of several normal and cold acclimated mussels was estimated by the spectrophotometric method of Spirin (*9*). The concentration of nucleic acid phosphorus in the hepatopancreas of cold acclimated mussels doubled compared to normal, but in the foot and ctenidia the increase was not so noticeable. These results show that metabolically active tissue (hepatopancreas) exhibits a great increase in nucleic acid content, implying increased protein synthesis, which is also indicated by the increased protein content of the cells.

The sequence of systemic changes, increased protein synthesis, and increased nucleic acid recall the sequence of events preceding molting of insects. Because the process in insects is triggered by hormonal agencies arising in neurosecretory cells, an attempt was made to localize such agencies in earthworms. The supra- and subesophageal ganglia of normal and cold earthworms were sectioned at 6 μ in paraffin and stained in Gomori's chrome alum haematoxylin-phloxine to show neurosecretory cells. As judged by the intensity of staining and dense packing of particles, there is a distinct increase in the activity of neurosecretory cells in cold acclimated individuals. This increase suggests the possibility that a hormonal product of neurosecretion plays an important role either by triggering the events that cause metabolic compensation through systemic changes and increased protein synthesis, or by directly influencing tissue metabolism,