The Campaign for Nuclear Disarmament in Britain recently invited scientists to a meeting to discuss the part they could play in diminishing the danger of nuclear war. Bertrand Russell, J. Rotblat, and John Collins, chairman of the campaign, spoke on what scientists had done and must do in the present dangerous situation. The meeting set up a working party to formulate policy and action. The chairman is N. Kemmer, and the secretary, A. Pirie, The Ford, North Hinksey Village, Oxford, England. The group of scientists hope to cooperate with scientists in other countries and would be glad to hear from such groups.

for a man of science to say with any honesty, "My business is to provide knowledge, and what use is made of the knowledge is not my responsibility." The knowledge that a man of science provides may fall into the hands of men or institutions devoted to utterly unworthy objects. I do not suggest that a man of science, or even a large body of men of science, can altogether prevent this, but they can diminish the magnitude of the evil.

There is another direction in which men of science can attempt to provide leadership. They can suggest and urge in many ways the value of those branches of science of which the important practical uses are beneficial and not harmful. Consider what might be done if the money at present spent on armaments were spent on increasing and distributing the food supply of the world and diminishing the population pressure. In a few decades, poverty and malnutrition, which now afflict more than half the population of the globe, could be ended. But at present almost all the governments of great states consider that it is better to spend money on killing foreigners than on keeping their own subjects alive. Possibilities of a hopeful sort in whatever field can best be worked out and stated authoritatively by men of science; and, since they can do this work better than others, it is part of their duty to do it.

As the world becomes more technically unified, life in an ivory tower becomes increasingly impossible. Not only so; the man who stands out against the powerful organizations which control most of human activity is apt to find himself no longer in the ivory tower, with a wide outlook over a sunny landscape, but in the dark and subterranean dungeon upon which the ivory tower was erected. To risk such a habitation demands courage. It will not be necessary to inhabit the dungeon if there are many who are willing to risk it, for everybody knows that the modern world depends upon scientists, and, if they are insistent, they must be listened to. We have it in our power to make a good world; and, therefore, with whatever labor and risk, we must make it.

Molecular Heterogeneity and Evolution of Enzymes

Coenzyme analogs are useful for studying the evolution, classification, and differentiation of enzymes.

Nathan O. Kaplan, Margaret M. Ciotti,
Milton Hamolsky, Robert E. Bieber

In recent years the development of new techniques has led to awareness of the heterogeneity of proteins which serve the same function. The classic studies on the hemoglobins, in particular sickle-cell hemoglobin, have clearly illustrated the use of chromatographic and electrophoretic techniques in establishing molecular heterogeneity. The immunological approach to detecting similarities and dissimilarities of enzymes has also attracted much interest. For example, immunological techniques have been used to identify differences between muscle and liver phosphorylases (*1*). Schlamowitz (*2*) has shown differences in alkaline phosphatases by immunological methods. Physical properties have also been used to identify differences between proteins catalyzing the same functions. Crystalline yeast alcohol dehydrogenase can be distinguished from horse-liver alcohol dehydrogenase simply by the fact that its molecular weight is almost twice that of the liver enzyme (*3*).

Another method of detecting the molecular heterogeneity of proteins is the determination of amino acid sequence. Although hormones from different sources may differ somewhat in amino acid sequence, they appear to have the same general physiological properties. Sanger (*4*), in his work on insulin, has shown that the hormone isolated from one species may differ slightly in amino acid sequence from that isolated from another. Similarly, studies with adrenocorticotropic hormone have shown that pig, lamb, and beef hormones have different amino acid sequences (*5*).

Although there has been some investigation of the heterogeneity of enzymes that catalyze the same function, the approach of differentiating the enzymes by their catalytic activities has not been fully exploited. It is the purpose of this article to present evidence that the catalytic technique may be most useful in discriminating differences between enzymes having the same function. Furthermore, data are given showing that this type of approach may prove to be a useful adjunct in studies of the ontogeny of enzymes as well as in studies of the genetic aspects of enzyme formation.

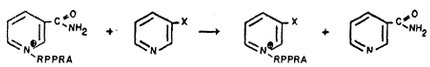
Dr. Kaplan, Miss Ciotti, and Mr. Bieber are affiliated with the graduate department of biochemistry, Brandeis University, Waltham, Mass. Dr. Hamolsky, who was formerly affiliated with the department, is now on the staff of Beth Israel Hospital, Boston, Mass.

Diphosphopyridine Nucleotidases

Some years ago we reported that isonicotinic acid hydrazide (INH) strongly inhibited several animal-tissue diphosphopyridine nucleotidases (*6*). Such inhibition was found to be characteristic of the ruminants. Table 1 lists the animals whose tissue diphosphopyridine nucleotidases are "sensitive" or "insensitive" to isonicotinic acid hydrazide. It is of interest that the diphosphopyridine nucleotidases in all the tissues of the "sensitive" animals are inhibited by a low concentration ($7.5 \times 10^{-4}M$) of this 4-substituted pyridine derivative, whereas, in the tissues of the "insensitive" animals, they are inhibited to a much lesser degree by the same concentration of this compound. Thus, the degree of sensitivity to isonicotinic acid hydrazide and other 4-substituted pyridine compounds (*7*) appears to be a specific characteristic of the tissue diphosphopyridine nucleotidases of the suborder of ruminants. This would suggest that whereas the diphosphopyridine nucleotidases from the different species in the suborder are closely related, they differ in their properties from those of other mammals.

Coenzyme Analogs and Molecular Heterogeneity

Although a number of enzymes are components of all tissues, the question arises as to whether the enzymes from different tissues of the same species are identical. During the past few years we have been approaching this problem through the use of analogs of diphosphopyridine nucleotide (DPN). These are pyridine-substituted analogs (Fig. 1). They have been prepared by an exchange reaction catalyzed by the animal-tissue diphosphopyridine nucleotidase, in which a pyridine compound replaces the nicotinamide moiety (*8*, *9*):



where *A* is adenine, *R* is ribose, and *P* is phosphate. The deamino DPN, which is the hypoxanthine analog of DPN, can be obtained by treatment of the natural coenzyme with nitrous acid (*10*).

In our early work on the coenzyme analogs (*11*) it was observed that crystalline lactic dehydrogenases from beef heart and rabbit skeletal muscle appeared to act differently with several coenzyme analogs. This led us to investigate in some detail whether this difference was due to the fact that the enzymes were obtained from two different animals or to the fact that two different tissues were involved.

Before data are presented on the various lactic dehydrogenases, it is important to discuss briefly some of the characteristics of the interaction between substrate and coenzyme with enzymes such as lactic dehydrogenase. It appears that the mechanism of the reaction may involve a ternary complex between the enzyme, the substrate, and the coenzyme. This is indicated by the different affinity constants obtained with substrates when different coenzymes are used, as illustrated in Fig. 2 for beef-heart lactic dehydrogenase, where the saturation curves of pyruvate with reduced diphosphopyridine nucleotide (DPNH), deamino DPNH, and reduced triphosphopyridine nucleotide (TPNH) are shown.

For example, it takes much higher concentrations of pyruvate to saturate the reaction with TPNH than with either DPNH or deamino DPNH. Furthermore, one of the characteristics of the kinetics of lactic dehydrogenase reactions is substrate inhibition, and it may be seen from the curves in Fig. 2 that pyruvate at high concentrations inhibits the rate of reaction with both DPNH and deamino DPNH. The figure also shows that substrate inhibition is dependent on the hydrogen acceptor used. Thus, the group of dehydrogenases of the lactic acid type shows different substrate saturation curves with different coenzyme analogs, and also shows the general property of substrate inhibition. It is important to stress that substrate inhibition and saturation with different coenzyme analogs appear to vary quantitatively with different lactic dehydrogenases.

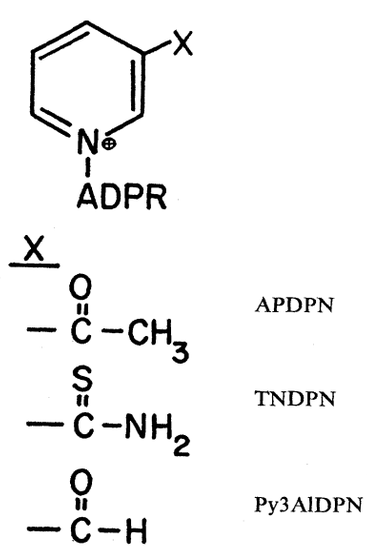


Fig. 1. Pyridine-substituted analogs of diphosphopyridine nucleotide.

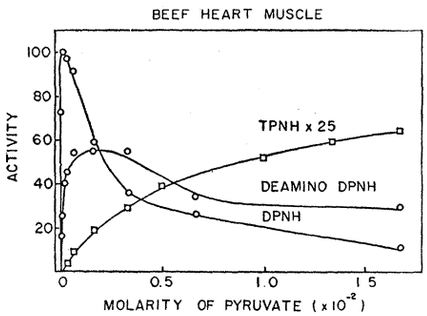


Fig. 2. Saturation of beef heart lactic dehydrogenase with different concentrations of pyruvate, different reduced pyridine coenzymes being used. Twenty-five times as much enzyme was used with the TPNH as with the DPNH or the deamino DPNH.

In order to distinguish differences between the lactic dehydrogenases, we have utilized several types of catalytic data. For assay of the enzymes, several concentrations of lactate or pyruvate were used with various analogs as well as with the natural coenzymes. In general, two concentrations of either lactate or pyruvate were used. They are referred to in this study as *H* (high concentration, equivalent to $0.1M$ lactate and $3 \times 10^{-3}M$ pyruvate) and *L* (low concentration, equivalent to $0.013M$ lactate and $3 \times 10^{-4}M$ pyruvate). For example, DPN_H symbolizes the rate of reaction with the high concentration of lactate when DPN is the coenzyme; $APDPN_L$ indicates the rate with the low level of lactate and the acetylpyridine analog as coenzyme. The terms $DPNH_L$ and $DPNH_H$ represent the rates with the low and high levels of pyruvate, respectively, when the reduced DPN is the hydrogen donor. The reactions were all carried out with an

Table 1. The effect of isonicotinic acid hydrazide (INH) on the diphosphopyridine nucleotidases of various animals. The action of all the "sensitive" enzymes was inhibited to an extent greater than 65 percent by $7.5 \times 10^{-4}M$ INH; the action of the "insensitive" enzymes was not significantly inhibited by $7.5 \times 10^{-4}M$ INH.

INH-"sensitive"	INH-"insensitive"
Goat	Pig
Beef	Horse
Lamb	Mouse
Deer	Guinea pig
	Rat
	Rabbit
	Frog
	Man

excess of the pyridine nucleotides. Initial rates were determined; all determinations were made on a Zeiss spectrophotometer, the appropriate maxima for reduced DPN and its analogs being used (*9, 11*).

In general we have examined the activity of the various dehydrogenases in the soluble fraction of the tissues. After purification or crystallization, the same relative results are obtained as with the soluble fraction. This has been found with enzymes from human prostate gland, from beef heart, and from beef, rabbit, halibut, lobster, and *Limulus* muscle. In general, the values reported can be duplicated within a few percent when several animals of the same species are analyzed. It appears that the soluble fraction of the tissues can be used for the type of enzyme differentiation described below.

Table 2. The ratio of the reaction rates for lactic dehydrogenases from heart and muscle of different animals, with high and with low concentrations of pyruvate or lactate.

Animal	$DPNH_H/DPNH_L$		$APDPN_L/TNDPN_L$	
	Heart	Skeletal muscle	Heart	Skeletal muscle
Man	0.4	0.7	0.7	1.8
Mouse	0.5	0.8	1.2	3.0
Rat	0.4	0.8	0.7	2.4
Guinea pig	0.4	0.8	0.9	2.8
Rabbit	0.3	1.1	0.4	5.1
Beef	0.5	1.1	1.1	2.9
Pig	0.5	0.8	0.7	2.4
Lamb	0.4	1.2	0.7	3.5
Pigeon	0.2	0.7	0.8	3.0
Chicken	0.5	0.9	1.1	4.4
Bullfrog	0.7	0.8	0.7	4.9
Grass frog	0.4	0.7	0.5	4.5
Salamander	0.9	1.3	1.6	4.1
Box turtle	0.6	0.7	2.3	4.8
Painted turtle	0.8	1.0	2.8	9.0
Herring	1.2	1.9	2.9	11.6
Mackerel	0.9	3.2	0.8	11.5
Flounder	2.0	1.9	28	45
Sole	2.1	3.2	30	45
Halibut	*	1.9	*	49
Sea bass	0.9	1.9	0.9	5.6
Butterfish	0.8	1.7	0.8	10.6
Scup	0.6	1.4	0.6	4.9
Sea robin	1.1	1.4	1.9	8.5
Puffer	1.2	1.3	7.4	14.0
Toadfish	0.9	1.3	1.4	9.5
Suckerfish	0.5	1.0	0.9	6.0
Dogfish	0.4	1.1	1.2	8.0

* Not available.

Table 3. Comparison of the lactic dehydrogenase reaction rates of the thionicotinamide and acetylpyridine analogs of diphosphopyridine nucleotide in different human tissues.

Patient No.	$APDPN_L/TNDPN_L$			
	Heart	Liver	Kidney	Muscle
1	0.70	2.8	0.55	1.7
2	0.67	2.2	0.53	1.4
3	0.60	2.7	0.70	1.6
4	0.84	3.1	0.64	1.8

Table 4. Ratios of reaction rates of heart muscle lactic dehydrogenases in various species.

Item	Flounder	Sole	Lamb	Dogfish	Grass frog	Herring
$DPNH_H/DPNH_L$	2.0	2.2	0.4	0.4	0.4	1.1
$DeDPNH_H/DPNH_H$	0.5	0.7	2.7	2.9	1.8	0.95
DPN_H/DPN_L	3.3	3.5	1.1	1.2	1.1	1.9
$APDPN_L/DPN_L$	13.2	15.0	0.4	0.8	0.4	1.6
$APDPN_L/TNDPN_L$	28.0	30.0	0.7	1.2	0.5	2.9
$Py3AlDPN_L/DPN_L$	1.2	1.5	0.9	1.2	1.1	1.3
$Py3AlDPN_L/TNDPN_L$	2.5	3.0	1.6	2.0	1.3	2.1

Table 2 shows the ratio of the rates of reaction of lactic dehydrogenase from different sources when two different concentrations of pyruvate are used with a constant amount of DPNH. As can be observed, the ratios for the different mammalian heart enzymes are quite similar. The values indicate again that high concentrations of pyruvate inhibit the action of heart enzymes. There appears to be a distinct difference between the heart and muscle lactate dehydrogenases in the same species with regard to pyruvate saturation. It is of interest to note the similarity of findings for the bird- and mammalian-heart catalysts on the one hand and the skeletal-muscle enzymes on the other. Findings for enzymes in lower vertebrates appear to be somewhat different from those for enzymes in the higher forms, as is indicated particularly by the ratios obtained for different fish. The ratios reported in Table 2 for the fish-heart enzymes are in general somewhat higher than those for the higher vertebrates. This indicates that pyruvate at high concentrations is less inhibitory to the action of fish enzymes. The flounder and sole have particularly high ratios. Again, in the fish, there appears generally to be a difference between heart and muscle enzymes of the same species.

In Table 2 there is also a comparison of the activities of lactic dehydrogenase (LDH) employing the acetylpyridine DPN (APDPN) and thionicotinamide DPN (TNDPN) with low concentrations of lactate (*12*). It is evident from the data that the mammalian-heart enzymes are quite similar, and are different from the skeletal-muscle catalyst, with respect to their capacity to handle the two analogs. A comparison of these data indicates that there is a closer agreement among the heart lactic dehydrogenases from the different species than there is between the heart and the skeletal-muscle lactic dehydrogenases of the same species. The heart enzymes of the different mammals all have somewhat similar ratios. The ratios for pigeon and chicken enzymes, as well as for those of the two frog species studied, seem to be similar to those for mammalian proteins. There is a possibility that the ratios of the turtle enzymes may be somewhat different. However, as in the case of the changes in the DPNH pyruvate ratios, ratios for some fish demonstrate values distinctly different from those of the higher vertebrate enzymes. It is also of interest that the heart enzyme of the salamander appears to be different from that of the frog. Quite remarkable is the great difference observed in the group of flatfish—the sole, flounder, and halibut. The heart lactic dehydrogenases from the flatfish apparently utilize the acetylpyridine analog much better than they do the thionicotinamide analog; this is in complete contrast to the situation observed with the mammalian enzymes. The data in Table 2 clearly indicate that the flatfish have enzymes which react similarly, but these enzymes are distinctly different from those of other vertebrates studied.

In Man

We have obtained on autopsy several tissues from a number of human patients and have compared the lactic dehydrogenase activity of the various tissues by the coenzyme analog method. As may be seen in Table 3, enzymatic activities of the given tissues from the different individuals are in quite close agreement. It is evident from the ratios of reactivity with acetylpyridine DPN and thionicotinamide DPN that (i) the liver and muscle enzymes are distinct from the kidney and heart enzymes, and (ii) one can distinguish the muscle from the heart enzymes. However, the determinations do not appear to give a sharp differentiation between the kidney and the heart enzymes. We have further observed that with the catalytic response

to coenzyme analogs we can distinguish the lactic dehydrogenases of human spleen and lung from those of the four tissues listed in Table 3.

Recent data by Nisselbaum and Bodansky (*13*) indicate an immunological relation between rabbit tissue lactic dehydrogenases which is in general agreement with the results we have obtained in distinguishing the enzymes with the coenzyme analogs.

As a further approach to the classification of lactic dehydrogenases by their reaction rates, we have summarized in Table 4 some data obtained with different heart enzymes; these data show their reactions to various coenzyme analogs under different conditions. It is evident that enzymes of the flounder and of the sole are quite similar to each other, yet distinct from those of the other species listed. We feel that data of this type, which indicate similarities and dissimilarities with different analogs, are further evidence of the heterogeneity of the enzymes. We believe that the striking and consistent differences found in the reaction rates in flounder and sole represent differences in the enzyme proteins of these animals as compared to the enzymes of other species serving the same function. The heart lactic dehydrogenase of the dogfish seems more closely related to that of the grass frog and the lamb than to that of either the herring or the two flatfish. The enzyme of the herring has properties which are certainly distinct, and thus it is probably different from the enzymes of the other species listed. We would like to emphasize that at the present time we are stressing only large differences in ratios. It is possible that smaller differences in ratios are also significant—for example, a ratio of 0.8 in the dogfish as compared with 0.5 in the lamb. We feel, however, that much further work is required to show that such subtle differences are significant.

During the course of examining some of the fish, it was observed that the lactic dehydrogenases of certain species were different in the dark- and light-muscle extracts. The dark muscle in the fish was obtained from the dorsal surface of the animal, and in most cases it could be easily separated from the light tissue. Table 5 shows comparable data for a number of species with respect to lactic dehydrogenase from such dark and light muscles, as well as from heart muscle. For example, in the mackerel it is evident in nearly all the comparisons that the heart enzyme is very closely related to the dark-muscle enzyme and grossly different from the light-muscle enzyme. The trout is another example of a fish in which the enzyme from the light muscle differs greatly from the enzyme from the dark muscle. Here again the heart enzyme and the dark-muscle enzyme appear to be quite similar. In herring, butterfish, scup, and dogfish there is also a closer relationship between the dark-muscle and the heart enzymes than there is between the dark- and light-muscle enzymes. This finding suggests that the heart muscle and the dark muscle in these species may possibly have a common origin. On the other hand, the enzymes from the dark and light muscles of the sea robin and the salamander are very similar and can be distinguished from the heart enzyme. The lactic dehydrogenases from the dark and light muscles of the chicken are identical, according to our method of assay.

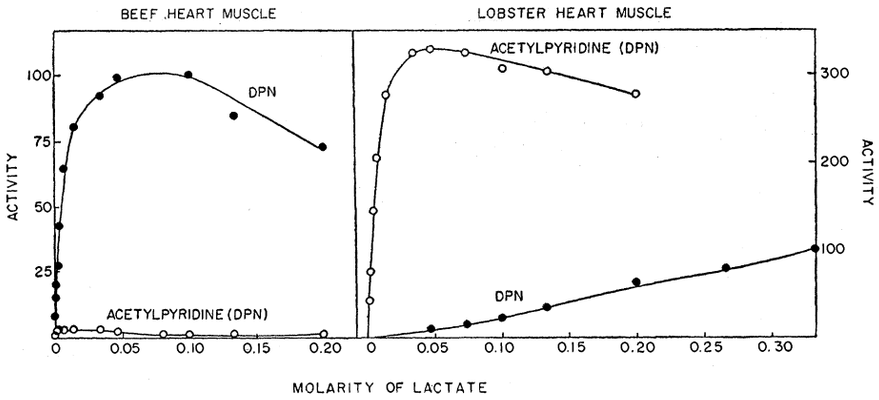


Fig. 3. Saturation of beef- and lobster-heart lactic dehydrogenases with lactate.

Vertebrates and Invertebrates

We also have made a preliminary survey of lactic dehydrogenases of invertebrates. There is in general a great difference between enzymes from invertebrates and vertebrates. Furthermore, the invertebrates show a great deal of interspecies diversification.

Figure 3 compares the lactic dehydrogenases from beef heart and lobster heart with respect to their reactivities to DPN and acetylpyridine DPN with different concentrations of lactate. The remarkable differences in reactivity certainly suggest that the two enzymes are very different. At low concentrations of lactate the lobster heart enzyme shows little or no activity with the natural coenzyme, in contrast to what is observed with the beef heart catalyst. If one measured the lactic dehydrogenase of lobster heart using DPN and the usual concentrations of lactate, one would conclude that there is relatively little or no enzyme in this tissue. How-

Table 5. Comparison of ratios of reaction rates for lactic dehydrogenase from heart with rates for dark and light muscle enzymes of different species.

Item	Ratios for LDH from muscle in											
	Mackerel			Trout			Sea robin			Salamander		
	Heart	Dark	Light	Heart	Dark	Light	Heart	Dark	Light	Heart	Dark	Light
$DPNH_H/DPNH_L$	0.9	0.9	3.2	0.9	0.9	2.5	1.1	1.7	1.4	0.9	1.3	1.1
DPN_H/DPN_L	1.5	1.5	2.7	1.6	1.9	3.2	1.3	2.9	3.2	1.5	2.1	1.9
$DeDPN_H/DeDPN_L$	1.3	2.0	3.9	2.0	1.7	3.9	2.9	4.6	4.1	2.8	3.2	3.1
$APDPN_L/DPN_L$	0.6	0.5	3.4	0.8	1.4	8.5	1.1	4.8	5.2	1.1	1.7	1.7
$APDPN_L/Py3AlDPN_L$	0.6	0.5	2.7	0.8	1.2	11.8	1.5	3.6	2.4	0.8	2.3	2.2
$APDPN_L/TNDPN_L$	0.8	0.6	11.5	1.5	2.3	23.6	1.9	10.5	8.5	1.6	3.9	4.1

Table 6. Ratios of reaction rates for lactic dehydrogenase for the phylum Arthropoda.

Source	$\frac{DPNH_H}{DPNH_L}$	$\frac{DeDPNH_H}{DPNH_H}$	$\frac{DPN_H}{DPN_L}$	$\frac{APDPN_L}{DPN_L}$	Detectable activity	
					TNDPN	Py3AlDPN
			Mandibulata			
Hermit-crab body muscle	1.3	0.9	9.4	28.0	+	+
Fiddler-crab claw muscle	1.8	0.2	>10	>200*	+	+
Green-crab body and claw muscle	1.7	0.3	6.0	>200*	+	+
Edible-crab body muscle	2.2	0.3	>10	>200*	+	+
Crayfish body muscle	2.8	0.2	>10	>200*	+	+
Lobster heart muscle	3.0	0.17	3.9	173	+	+
Lobster body muscle	1.2	0.7	9.0	85	+	+
Lobster claw muscle	2.0	0.3	4.0	200	+	+
			Chelicerata			
Limulus heart muscle	0.36	2.7	1.1	1.5	−	−
Limulus body muscle	0.59	1.8	1.5	0.4	−	−
Limulus liver	0.29	8.3	1.4	3.4	−	−
Tarantula muscle	0.5	1.1	1.3	0.6	−	−
Scorpion muscle	0.4	3.5	1.6	0.8	−	−
Wolf-spider muscle	0.35	1.8	1.5	0.3	−	Trace

* The activity with low concentrations of lactate and DPN was so low that it was impossible to obtain significantly accurate ratios.

ever, when one uses the coenzyme analog for measurement there seems to be a considerable concentration of this dehydrogenase. The enzymes appear to differ with respect to their turnover number when DPN is used as coenzyme. The lobster heart contains DPN, and there is no evidence that a pyridine nucleotide such as the acetylpyridine analog exists in the invertebrate. We have purified the lobster enzyme about 150-fold, and it is our impression that there is as much lactic dehydrogenase protein on a weight basis in lobster heart as there is in beef heart.

Lactic dehydrogenases from other tissues of lobster show the same relative low affinity that the heart enzyme shows for lactate with DPN as compared to acetylpyridine DPN. The affinity for DPN and lactate, however, seems to be somewhat greater for the enzyme from the thoracic muscle than for that from the heart. This is also illustrated by the lower $APDPN_L/DPN_L$ ratio (Table 6). There is also some difference in ratio between the enzymes obtained from the thoracic muscle and from the claw. Although lactic dehydrogenase activity has been found in the liver of *Limulus* (the horseshoe crab), we have not observed any significant activity in the corresponding organ of the lobster.

Other crustaceans, such as the hermit crab, the fiddler crab, the edible crab, and the crayfish, contain lactic dehydrogenases which have properties similar to those of the lobster. *Limulus*, however, which is related to the arachnids, appears to possess lactic dehydrogenases somewhat different from those of the crustaceans. For example, lactic dehydrogenase from either heart, body muscle, or liver of *Limulus* shows no significant reactivity with the pyridine-3-aldehyde and thionicotinamide analogs of DPN. The enzymes of *Limulus* react considerably better with DPN than does the lobster system, as is shown by the much smaller APDPN/DPN ratios given in Table 6. Furthermore, all the lactic dehydrogenases of *Limulus* studied show a relatively low affinity for lactate when the acetylpyridine analog of DPN is used. This is in contrast to other species where the system is saturated with much lower concentrations of lactate when acetylpyridine DPN is the coenzyme. The inactivity with the pyridine-3-aldehyde and thionicotinamide analogs, as well as differences in lactate saturation with the acetylpyridine DPN, appear to be characteristic of the lactic dehydrogenases of *Limulus*. Further distinguishing differences between the lobster and *Limulus* are given in Table 6. The difference in reaction to increasing concentrations of pyruvate ($DPNH_H/DPNH_L$), as well as the ratios with deamino DPNH, clearly indicate that the enzymes from the two invertebrates are different.

Scorpions, spiders, and tarantulas are in the same subphylum as *Limulus*, and extracts of these animals have given lactic dehydrogenases which have properties somewhat similar to those of the enzymes of *Limulus*. The chelicerate enzymes do not react with the thionicotinamide and pyridine-3-aldehyde analogs and show a remarkably low affinity for lactate in the presence of acetylpyridine DPN. Although lactic dehydrogenases from different tissues of *Limulus* appear to have some similar properties, there are characteristics which distinguish the enzymes from individual tissue (Table 6). Such evidence suggests that the lactic dehydrogenases of one species may have common properties which are modified in the different tissues. Lactic dehydrogenases from squids, leeches, shrimp, and mollusks all appear to be distinct from one another and different from those of the animals listed in the table.

Although the lactic dehydrogenases appear to be quite heterogeneous as determined by the coenzyme analogs, we have found it somewhat more difficult to show differences in malic dehydrogenases. In contrast to the results with the lactic dehydrogenases, malic dehydrogenases from human heart and muscle are found to be quite similar. Furthermore, they are not very different from the corresponding enzymes from, for example, *Limulus*. On the other hand, certain of the malic dehydrogenases have shown great differences. This has been particularly true of the enzymes from clam and halibut muscle.

Markert and Møller (*14*) have recently reported differences in lactic dehydrogenases in different tissues, determined by electrophoretic techniques. We have also found that, in agreement with the observations with coenzyme analogs, the muscle and heart enzymes can be distinguished by their electrophoretic mobilities. In contrast, lactic dehydrogenases from lobster muscle and rabbit skeletal muscle move almost identically on electrophoresis. However, they are different catalytically and immunochemically. An antibody to the lobster muscle enzyme has been found to cross-react with certain crab enzymes but not with the *Limulus* enzymes.

Bueding and his associates (*15, 16*) have obtained some kinetic and immunologic data indicating that the lactic dehydrogenases of *Schistosoma mansoni* and rabbit muscle are different. The antibody to *S. mansoni* will inactivate the worm enzyme but not the rabbit muscle enzyme (*16*). Furthermore, it was found that the antibody obtained from one *Schistosoma* species will cross-react with enzymes from other *Schistosoma* species.

Recent studies by Elödi (*17*) have shown that glyceraldehyde-3-phosphate dehydrogenases from the muscles of several mammals (rabbit, swine, and bovine) are identical with respect to physical, chemical, catalytic, and immunological properties. The enzyme isolated from crayfish, however, appears to differ with respect to these characteristics from the mammalian enzymes.

Summary and Conclusions

We feel that the observations described here indicate the molecular heterogeneity of the lactic dehydrogenases obtained from different tissues and from different animals. The data presented indicate that it is possible to classify animals not only by their physiological and morphological characteristics but also by their enzymatic properties, and they also suggest that change in enzyme structure may have been of significance in the establishment of new species. It is also our impression from this preliminary survey, that lactic dehydrogenases of the heart may have undergone considerably greater evolutionary changes than those of the skeletal muscles.

We believe that the results outlined in this article may be of value in studying the interrelationship and origin of species. This may be of particular importance in connection with the flatfish, since the origin of this group of fish is at present quite unresolved. We hope our investigations will eventually enable us to ascertain whether certain peptide chains have been altered during species evolution, as well as in individual differentiation. It is evident from the data presented that the coenzyme analogs (some of which have been used previously and some of which have been recently synthesized) are valuable adjuncts in detecting the heterogeneity of dehydrogenases. It is our hope that studies with crystalline dehydrogenase may enable us to ascertain what part of the enzyme molecule is undergoing structural change during evolution (*18*).

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12. Since most of the analogs react more slowly with the lactic dehydrogenases than with the natural coenzyme, the reactions were carried out with different levels of enzyme. This was particularly true with the mammalian systems. The data in the table represent five times the actual reaction rate observed with acetylpyridine DPN and pyridine-3-aldehyde DPN and twice the actual rate with thionicotinamide DPN. The ratios for deamino DPN and deamino DPNH were obtained from the actual rates. For purposes of comparison, this system has been maintained even in those cases where the reactions are faster with the analogs than with the natural coenzymes—that is, with enzymes from invertebrates.
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L. V. Heilbrunn, General Physiologist

An Outline of General Physiology appeared first in 1937. Like its author, the book was definitive, far-ranging in scope, imaginative, and provocative. Lewis Victor Heilbrunn was certainly one of the most influential individuals in general physiology during the last four decades. Through his book on general physiology (which was thoroughly revised in 1943 and 1952), his scientific papers, his monographs, and most especially his many devoted graduate students, his influence was felt throughout the scientific world. His untimely death in an automobile accident on 24 October snuffed out a creative spirit science can ill afford to lose, but his influence will continue for generations to come.

L. V. Heilbrunn was born in Brooklyn, New York, 24 January 1892. He attended Cornell University as an undergraduate and was awarded the Ph.D. under the direction of Frank R. Lillie at the University of Chicago in 1914, at the age of 22. He then taught for a brief period at the University of Illinois Medical School and for 8 years at the University of Michigan, and he spent a year abroad as a Guggenheim fellow. In 1929 he was appointed to the staff of the department of zoology, University of Pennsylvania—an association that continued for 30 years, until his death.

At Pennsylvania he helped to organize, and became chairman of, the graduate department of general physiology. His course in general physiology was given to senior premedical students and biology majors as well as to graduate students in the department of zoology, and through his course he had a marked influence on many physicians and on scientists in other fields of biology. The course was always popular and well attended and, as one of its most interesting features, the students were given research projects during the second semester rather than formal laboratory work.

Well over 50 scientists took their Ph.D. degrees under Heilbrunn's direction, and many others did part of their graduate work with him. This group, with its members located all over the United States and abroad, probably represents the most scientifically productive group in general physiology in America to be trained by one individual. Their influence on biology in this country is undoubtedly immense.

Heilbrunn's research interests ranged widely, but with a central theme of protoplasmic structure and action. At a time when general physiology tended to be preoccupied with membranes and permeability, he pioneered in focusing attention upon actions within the body of the cell. His views were first summarized in *The Colloid Chemistry of Protoplasm* in 1928, a book which laid the foundation for an important aspect of cell physiology and which evoked discussion for many ensuing years. One of the most important and influential