Variety of cane used	Number of experiment and of cane	Number of seed piece										
		Checks uninoculated		Inoculated								
		a	b	1	2	3	4	5	6	7	8	9	10	11
Foenix[2]	VI– 5	0	0	?	x	x	?	?	x	x				
Foenix	VI– 6	0	0	x	x	x	x	?	x	x	x			
Foenix	VI– 9	0	0	x	died	?	x	x	x	x	x	x		
Cavengerie	VIII–3	0	0	x	x	x	x	x	x	x	x	0	0	0

active for various days, even though this tissue is not, at the time, in a process of active growth.

In the accompanying table (where *0* corresponds to no infection, *x* to unquestionable and *?* to questionable infection) are reported some of the results secured in our work, the final readings being taken two months after inoculation in the case of the "Foenix" cane, and one month and ten days after inoculation in the case of the Cavengerie. The plants were grown, in this case, in an insect-proof cloth house with frequent preventive tobacco fumigations.

AUGUSTO BONAZZI

ORIENTE, CUBA

POST-LARVAL LOBSTERS

MUCH has been learned from time to time about the life habits of adult lobsters. This is also true of lobster eggs and of young fry. It is true, however, that very little has been known about young lobsters one, two and three years of age. These animals are very seldom seen. Many lobster fishermen, for example, have never seen a two-inch lobster.

During the two summers of 1919 and 1920 the writer undertook the work of capturing some of these very young lobsters. Accompanied by my son I began testing out all sorts of places in Richmond Bay, Prince Edward Island. This body of water was selected because the water is warm and shallow and the bay is well protected in every way.

Various methods for capturing lobsters were tried. First we tried the use of small traps. These were miniature models of the regular parlor traps used by fishermen, with the exception of a few, which consisted of only one compartment.

The traps were baited and set out in water varying in depth from two to ten feet at low tide. They were put in rocky, sandy, muddy and grassy places. Quite a number of lobsters under five inches were caught in this way, one of which measured two and one half inches in length.

We also worked with a beam trawl when the weather was favorable and obtained good results in places where the bottom was soft, and covered in spots with short eel grass. A good deal of time was spent walking along shore on fine days when the water was at low tide. One day, by great good fortune, we found a place where there were some holes in the soft bottom of the ocean. After investigating many of these openings we succeeded at last in locating several small lobsters hiding in these "burrows." It was also observed that some burrows had two openings, an entrance and an exit. In such cases the openings were from six to fifteen inches apart. The lobster was therefore prepared to use either opening when attacked by an enemy.

By using all the methods indicated above we captured 280 lobsters six inches and under, 154 lobsters five inches and under, fifty-four lobsters four inches and under and four lobsters three inches and under. The smallest measured two and one half inches in length.

All lobsters taken were examined, measured and recorded and afterwards liberated away from the places from which they were obtained.

The investigation proved two things: first, that there are certain natural breeding grounds for lobsters; and, secondly, that young lobsters hide in all sorts of places, under rocks, in grass and even in burrows.

So far as I know this is the first authentic record of lobsters actually found living in burrows.

The expense of this investigation was borne by the Biological Board of Canada, and the work was done under the supervision of the chairman, Dr. A. P. Knight.

D. A. MACKAY

COLLEGIATE INSTITUTE, OTTAWA

[2] Foenix is the provisional name given to a cane, received from the Foenix gardens in Habana, which closely resembles D 74.

THE NATIONAL ACADEMY OF SCIENCES

(Continued from page 510)

Observations on the nature of ossification: W. G. MACCALLUM.

Bone is formed in the cartilage along the line of ossification of the epiphysis, but not in that of the ears or

the rings of the trachea. Various explanations have been offered, such as differences in carbon dioxide tension, physical characters, etc., but none are satisfactory. There is iron in the cartilage at the line of ossification, as shown in 1902 by Gierke, but none in the non-ossifying cartilages. It seems possible that this determines the disposition of calcium phosphate in that line.

We have found that the presence of the iron is perhaps due to a special affinity of the matrix of the line of ossification for ferric salts, since it absorbs them from a solution, in sharp contrast with other cartilage. It has long been known that pathological calcification is usually, if not always preceded by a deposition of iron. Tissues artificially impregnated with iron and suspended in solutions of calcium and phosphates become loaded with calcium phosphate more readily than those which contain no iron.

If a series of test tubes be set up, with constant quantities of calcium and increasing concentrations of phosphates, or vice versa, and kept at constant reaction, precipitation will occur after 12–24 hours at that point at which the concentration is sufficient. If these solutions are left standing long enough, precipitation gradually appears at less and less concentration, and would probably appear in the course of years in the most dilute.

If a trace of iron salt be added to each of a similar series of tubes, precipitation occurs far more rapidly, so that in a given time it appears in mixtures of far less concentration than those in which it takes place spontaneously. The iron seems to affect the velocity of the reaction rather than the mass action.

Epidemic encephalitis and simple herpes: SIMON FLEXNER.

The death rate from diphtheria in Massachusetts for 51 years, 1875–1925: CARL R. DOERING. (Communicated by Edwin B. Wilson.)

Thyroid and parathyroid in the chemical differentiation of bone: FREDERICK S. HAMMETT (introduced by Henry H. Donaldson).

Bones grow by increments of water, ash and organic matter. The percentage of water decreases, while that of organic matter and ash increase with the progress of growth. The decrease in ash percentage is largely due to ash deposition. In conditions of thyroid and parathyroid deficiency, bones grow less rapidly than normal. This reduction is not a simple proportionate affair in which the three constituents studied participate in equal degree, but is disproportionate. This indicates a distortion of the normal relations. The bones of the thyroidless rats show a definite tendency towards a lesser percentage of water, and a higher percentage of ash and organic matter than is to be expected for the bone weight. In this sense ossification is apparently not abnormally retarded in conditions of thyroid deficiency. The normal reciprocal relation between water and ash is, however, disturbed. In the bones of the parathyroidless rats a different situation is found. The normal reciprocal relation between water and ash is maintained. In the bones of the female there is a very evident tendency towards a lower percentage of ash and a higher percentage of water than that to be expected on the basis of actual bone weight. This indicates a retardation of ossification. In the male this trend is neither marked nor consistent. The greater sensitivity of the female is likewise exhibited in the thyroidless rats. In both sexes parathyroid deficiency produces no significant distortion of organic matter percentage. It is clear that the two types of glandular deficiency produce two distinct types of distortion in the chemical differentiation of bone during growth. It is probable that the chief factor in the production of the observed end results in the thyroidless groups is the disturbance of the water exchange of the organism, a phenomenon which has been observed in the blood and central nervous system of these same animals. In the parathyroidless groups it is probable that the disturbance of the calcium metabolism is the initiating factor in the distortion. In this study the humerus and femur of 135 rats thyro-parathyroidectomized at 23, 30, 50, 65, 75, and 100 days of age and allowed to grow until 150 days old, and their litter controls were analyzed, and those of 161 rats parathyroidectomized at like ages and kept under like conditions of diet and environment with their litter mate controls. All the rats were healthy and free from vermin. They had a common inheritance, being the descendants of two pair.

The permeability of mammalian erythrocytes and certain other cells to ammonium salts: M. H. JACOBS (introduced by C. E. McClung).

The recent studies of Michaelis on the properties of artificial membranes permeable to ions of one sign of charge and not to those of the opposite sign might be applied to the mammalian erythrocyte except for the fact that these bodies are generally believed to be permeable not only to anions but also to the two cations, H and NH_4. It has been pointed out by others that apparent permeability to H ions might equally well be explained as due to permeability to OH' ions. The case of the NH_4 ion which therefore presents the most serious remaining difficulty is that here considered.

Evidence is given that an ammonium salt such as NH_4Cl gains access to the erythrocyte by the penetration of undissociated ammonia followed by an exchange of OH' and Cl' ions. The ammonium as such does not appear to enter. In cells where an exchange of anions can not occur the peculiar penetrating power of ammonium salts largely disappears. The egg of Arbacia has been used for purposes of comparison with the mammalian erythrocyte. Finally, studies on the nucleated erythrocytes of a fish show that they resemble in this regard other nucleated cells rather than mammalian erythrocytes.

Quantitative potentiometric measurements on intra-cellular pH values of single fundulus egg cells: J. H. BODINE (introduced by C. E. McClung).

Studies on internal reactions of cells as well as permeability of cells to acid and chemicals have usually been concerned with changes in the color of intra-vital indicators introduced or taken up by the cell as well as changes in naturally occurring cellular pigments. Mortality and other functional changes have also been used as criteria of penetration of chemicals into cells. In all of these methods no accurate quantitative measurements of intracellular changes are obtained due to the inherent errors and limitations in such procedures. Data obtained by these methods, however, show intra-cellular reactions to be normally of an acid nature.

By means of an especially devised micro-hydrogen electrode and vessels, requiring extremely small quantities of fluid (0.01 cc.), it has been found possible to satisfactorily measure in an accurate quantitative way the intracellular reactions (pH) of single egg cells of the marine fish, *Fundulus heteroclitus*. Three separate determinations can be made on a single egg cell. Both fertilized and unfertilized egg cells were used.

The internal reactions (pH) of unfertilized egg cells, as determined when cells are taken directly from the female, are acid; an average pH of 6.39. Upon standing in sea water for periods of 72 hours or longer the unfertilized eggs become more acid in reaction. Variations in the internal pH values of unfertilized eggs have been found to be due to differences in the age of the eggs as taken from the female. Fertilized eggs also show an internal acid reaction, an average pH of 6.39, as well as a much less greater degree of variation than shown by unfertilized eggs of different ages. No marked changes in internal reactions of the eggs were detected during the course of development of the embryo.

It is of some interest to note that *Fundulus* eggs, normally living in sea water and exposed to fairly high alkaline reactions (pH 8.2), show a marked intra-cellular acidity (pH 6.39).

Rates of penetration of acid (HCL) into the eggs of *Fundulus* were also studied by means of this method. The extremely high resistance of the egg to acid is strikingly shown by the fact that considerably long exposures to acid (HCL-pH 4.3) are necessary before any appreciable change in internal pH takes place. It has also been found that cessation of heart beat and other functional changes in the embryo are not necessarily correlated with changes in the internal pH of the egg cell.

Suggestions regarding protoplasmic surfaces: W. J. V. OSTERHOUT.

By employing large multinucleate cells (one to six inches in length) certain experiments can be performed which are impossible with cells of ordinary size. Use has been made of this in the study of protoplasmic surfaces. In the case of *Valonia macrophysa* it is found that few salts penetrate and these with great slowness. This could be accounted for by supposing that the outer surface of the protoplasm permits little or no penetration of ions but allows certain undissociated molecules to pass. This agrees with experiments on electrical conductivity (made with direct current) and with measurements of the potential differences across the protoplasmic layer. It is not certain that no ions enter the protoplasm as such, but if ions enter it must be to a very slight extent or else their relative mobilities must be greatly reduced.

It seems probable that selective permeability may be exercised with respect to undissociated molecules rather than with respect to single ions (its seat may be at the external surface or deeper in the protoplasm). If selective permeability were exercised with respect to single ions we should expect salts which penetrate to give a different P. D. from those which do not. This is in general not the case in *Valonia*, at least not to any marked extent.

In the case of *Valonia* contact may be made with the interior of the cell by means of a glass capillary (filled with sap) which is inserted through the protoplasm into the vacuole. This apparently permits us to approximate the absolute values of the P. D. across the protoplasm at any point where an external contact is made. In the case of Nitella such absolute values may be approximated by other methods.

It may also be possible to obtain approximate values for the absolute P. D.'s of the inner, and likewise of the outer, protoplasmic surface under certain conditions.

The physical properties of erythrocytes: WILLIAM SEIFRIZ (introduced by Henry H. Donaldson).

The red corpuscle of amphibians is a disk-shaped cell containing a huge nucleus, and a thin layer of cytoplasm (haemoglobin) enclosed by a resistant pellicle. The cell as a whole is quite elastic. It can, with the aid of micro-needles, be stretched to four times its length. The pellicle may exhibit highly elastic qualities or at other times be more plastic. It, like all cell membranes, is but a more concentrated layer of the protoplasm, capable of undergoing the same changes in physical properties that any protoplasmic mass undergoes. The cytoplasmic content of the corpuscle is a more fluid substance. The nucleus is of high viscosity and extraordinarily elastic. A liberated nucleus 14 μ in diameter may be stretched to a fine thread 350 μ long, and on release of the micro-needles the nuclear material returns to nearly its original size. The human erythrocyte exhibits physical properties similar to those of amphibians in regard to the cell membrane and the haemoglobin-containing contents. The 8 by 9 μ human corpuscle is a biconcave disk which may assume the form of an invaginated sphere or a cup. The cell as a whole is usually plastic, but may exhibit marked elastic qualities. The high elasticity of protoplasm points conclusively to a fibrous and possibly crystalline structure, that structure which is characteristic of all proteins.

If we are justified in assuming the presence of an ultimate living substance in cells that substance is certainly protein in nature.