Earlier workers used Bohr's value of $\alpha = 0.541$ at 38° in which case $c = 10^{-1.495}$, whereas most recent workers have used Van Slyke, Sendroy, Hastings and Niel's[6] value of α of 0.51 at 38°, in which case $c = 10^{-1.92}$. Hastings, Sendroy and Van Slyke[7] reviewing recent literature found pk′ averaged 6.104, using Bohr's α, and 6.13, using their own value of α. *In either case* $pk_2 = 7.625$. If, however, the value given in their summary of $pk' = 6.10$ is used, pk_2 becomes 7.62. *Since the value of* α *is not the same for all serums it seems of advantage to use* k_2 *in place of* $k'c$ *and plot quantities that can be directly determined: i.e.,* pH, $p\mathrm{CO_2}$ and [$\mathrm{BHCO_3}$]. Hence I have redrawn my log-log-pH paper with $k_2 = k'c$ of Hastings, Sendroy and Van Slyke, and reproduce it here (Fig. 1).

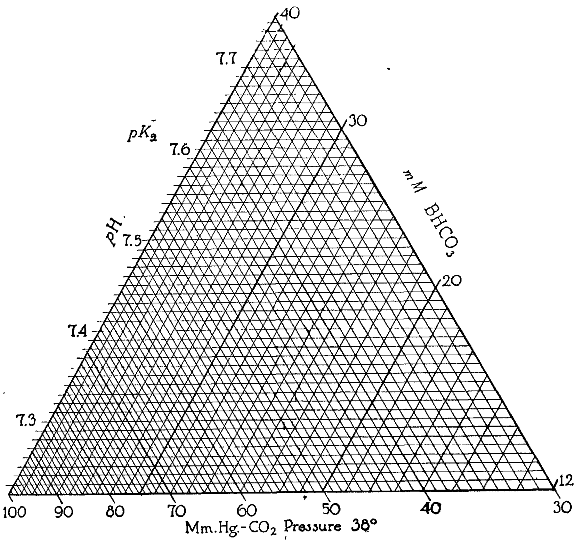

FIG. 1.

Since at all points where $p\mathrm{CO_2} = [\mathrm{BHCO_3}]$, $p\mathrm{H} = pk_2$, I have marked that value on the pH scale. In case any other value of pk_2 is considered more correct it is only necessary to slide the numbers up or down the pH scale until the pk_2 line corresponds to its new value.

On this graph paper it is easy to mark what takes place during a respiratory cycle or during acidosis or alkalosis, both compensated and uncompensated. In health and comparative rest the values of the blood fluctuate around the center of the chart, being in the arteries above and to the right of the center and in the veins below and to the left of the center. Under extreme conditions the values may go beyond the range of the graph. For example, in order to remove the compensating action of the respiratory center a cat was put under artificial respiration, and when this was markedly increased, the values for arterial blood moved off the graph upward and to the right, whereas the values for the veins remained nearer the center. When the maximum rate of the artificial respiration apparatus was reached an attempt was made to blow more $\mathrm{CO_2}$ out of the blood by removing the surface layer from the base of the lungs with sandpaper and blowing a continuous stream of air through the lungs. The same result was obtained, the venous blood remaining near its normal value. The explanation of this was found in observing the output of the heart. When the respiratory center was put out of action the center or centers controlling the circulation (vasomotor and vagus centers?) regulated the blood and, although the arterial blood was very deficient in $\mathrm{CO_2}$, the blood moved so slowly through the capillaries that its normal $\mathrm{CO_2}$ content was restored.

Since the arterial blood is spread over 125 sq. m. of surface in contact with alveolar air in the lungs, it is safe to assume that the $\mathrm{CO_2}$ partial pressure in the alveolar air is as close to that of arterial blood as could be determined in any ordinary apparatus. Although the partial pressure is not uniform in the different alveoli, the mixed alveolar air should be very close to the mixed arterial blood in $\mathrm{CO_2}$ partial pressure and hence at 38° these three values of pH, $\mathrm{CO_2}$ pressure and bicarbonate concentration may be determined in relation to the arterial blood taken from the living subject with precautions against loss of $\mathrm{CO_2}$ in the sample. In venous blood, however, it seems to me that the $\mathrm{CO_2}$ pressure is the most difficult to determine and it is better to determine pH and [$\mathrm{BHCO_3}$] and find $p\mathrm{CO_2}$ on the graph.

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SEXUAL PHASES IN PROSOBRANCH MOLLUSKS OF THE GENUS CREPIDULA

PROTANDRY, proterogyny, true hermaphroditism and, occasionally, self-fertilization have long been known to occur in Gastropods. In this group the species of *Crepidula* are of special interest, however, because in *C. plana,* which is normally protandric, it has been thought that the association of the young animal with an older individual, particularly one in the female phase, is essential for the development, as well as for the maintenance, of the functional male phase.[1] In another species, *C. fornicata,* which is likewise protandric, the length of time which the animal spends in the male phase was believed to be correlated with its continued opportunity for insemination.[2]

[5] Peters and Van Slyke, "Quant. Clinical Chem.," 1: 878, equation 11, 1931.

[6] *Jour. Biol. Chem.,* 78: 765, 1928.

[7] *Jour. Biol. Chem.,* 79: 183, 1928.

[1] Harvey N. Gould, *Jour. Exp. Zool.,* 23: 1–69; *Jour. Exp. Zool.,* 23: 225–250, 1917.

In both these species, as well as in *C. convexa,* nearly all individuals pass through a functional male phase while very young, often before the body has attained more than a small fraction of its normal definitive size, as was observed by Conklin many years ago.[3]

The male phase is followed by a series of transition stages, during which the long, muscular copulatory organ and the seminal vesicles are absorbed. Meanwhile the remaining spermatogenic cells of the gonad are cytolyzed, leaving only empty follicles with such ovogonia and ovocytes as were formed in the primary bisexual gonad of the very young animal. In the later transition period proliferation of ovogonia and growth of ovocytes accompany development of the uterus and seminal receptacles characteristic of the functional female phase, as Gould has so fully described.[1]

The two functional sexual phases are thus separated by a more or less extended transition period during which neither sex is dominant, since the animal returns essentially to a state of sexual immaturity. The phases are strictly progressive, however, for the transition gonad invariably develops into an ovary.

In some cases the male phase may be aborted, so that functional sexuality is not realized until the final, female, phase appears. Some individuals, too, show a tendency to remain in the male phase much longer than others. Since this strongly male characteristic is associated with smaller and fewer ovocytes in the gonad, it is thought to be due to a different combination of genetic factors than is present in animals which show a more active and briefer male phase.

This is not strictly a case of so-called "sex reversal"; it is merely the realization of the individual's genetic factors which lead first to the formation of the primary bisexual gonad, then to the functional male phase, followed by the transition stages and terminating in the full sexual maturity of the female phase.

No satisfactory evidence has been obtained to show that this sequence can be altered experimentally, although any one of the phases may be abbreviated or prolonged by various environmental conditions. Nor does it appear that in *C. plana* association of the young animal with older individuals, although usual, is essential for the realization of the functional male phase. Examination of hundreds of young individuals of that species which had attached themselves singly on dead shells of *C. fornicata* showed that such isolated young evidently become as fully functional males as do those which are associated with large females. Both the isolated and the associated young show much individual variability in the size that they reach before assuming the male phase, but the relative sizes are about the same in both environments.

The length of time that the male remains functional is, however, undoubtedly influenced by its environment, as Gould[1] and Orton[2] have observed. This fact is easily proved experimentally for *C. fornicata,* since in this species several individuals pile up in permanently attached groups, usually making a graded series, with the oldest at the bottom and youngest at the top. The oldest has, as a rule, reached the female phase, the younger transition phases and males being superimposed. If these groups be separated and the functional males isolated or segregated, the effects of the changed environment are very striking. Most of the males respond by promptly entering upon the transition stages which lead to the female phase. Spermatogenesis ceases, the penis and seminal vesicles are gradually absorbed and the spermatogenic cells are cytolyzed. Of more than 200 actively functional males which were thus segregated in June, 1934, about 15 per cent. had transformed to the female phase within 63 days; 39 per cent. had reached the third transition stage, 22 per cent. the second transition stage, 12 per cent. the first transition phase, while only about 11 per cent. had remained functionally male. Of an approximately equal number of males of similar sizes which had remained in their normal associations it was estimated that not more than 3 per cent. had reached the female phase during that time, and only 12 per cent. had begun the transition stages, while fully 85 per cent. still retained their function as males.

This experiment might be interpreted as indicating that the females in the intact groups exercise some restraining influence on the normal progressive change of sexuality of the males or that they in some way stimulate the continuation of the male's functions, but at least one other hypothesis should be considered. It must be remembered that each of the males in question has long since become so firmly attached to the shell of the underlying individual that movements are normally limited to merely raising or lowering the shell sufficiently to allow a circulation of the water needed for respiration and nutrition. When dislodged from their normal positions, however, the males struggle vigorously for hours or sometimes for days in efforts to right themselves and secure new attachments. Some of them later resume active locomotion. These active movements not improbably result in a more rapid metabolism which may conceivably initiate the first of the series of interdependent events leading to the sexuality of full maturity. The animal is thus prematurely aged in the sense that the sexual phase normally characteristic of an older age group appears when the body is less than half as large as it might otherwise have been at the beginning of the female phase.

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[2] J. H. Orton, *Proc. Roy. Soc. London,* 81B; 468–484, 1909; *Nature,* 110: 212–214, 1922.

[3] E. G. Conklin, *Jour. Morph.,* 13: 1–226, 1897; *Proc. Acad. Nat. Sci., Philadelphia,* 1898: 435–444, 1898.

Change of sex under these conditions is merely the premature realization of the animal's definitive genetic characteristics.

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PROTECTIVE VACCINATION OF HORSES WITH MODIFIED EQUINE ENCEPHALOMYELITIS VIRUS

BY serial passage through pigeons a strain of equine encephalomyelitis virus of the eastern type has been so changed that it promises to be of value as a vaccine. The pigeons were inoculated by the intracerebral route, under ether anesthesia, and the brain tissue for passage secured from birds that had just died or were killed when moribund. The virus has been carried through 100 passages, but most of the work to be reported was done with brains from the 40th and 49th serial passages.

In order to secure more material than is provided by the pigeon brain, a young lamb was inoculated intracerebrally with brain from the 40th pigeon passage and another lamb was likewise inoculated with brain from the 49th passage. Both animals promptly developed encephalomyelitis and died. Their brains were preserved in sterile 50 per cent. glycerin and suspensions were made as needed for the experiments. As little as 1 cc of a 10^{-3} dilution of a 10 per cent. suspension of the brain of either lamb injected subcutaneously into guinea pigs would immunize against from 10,000 to 100,000 infective doses of the unmodified virus injected either subcutaneously or intracerebrally. Of 117 guinea pigs inoculated with the 10 per cent. brain suspension, 8, or 7 per cent., died with symptoms of encephalitis and all but 15 of the remainder were immune. The majority of those that were not immune were tested by intracerebral injection of large amounts of virus. Had they been tested by the subcutaneous route they would probably have lived.

Although the modified virus usually fails to produce disease when injected subcutaneously, if it is brought directly into contact with the central nervous system an encephalomyelitis results. Its activity following intracerebral injection is, however, about 100 times less than that of the unmodified virus. Intracerebral passage of the modified virus through a horse, calf, sheep, rabbit, and serially through five guinea pigs has not restored the lost property of invasion of the central nervous system following subcutaneous injection.

Under controlled laboratory conditions 11 horses have been inoculated subcutaneously with suspensions of the lamb brains mentioned above. The majority of the animals were given 10 cc of a 10 per cent. suspension. Not one horse developed a temperature nor could virus be demonstrated in blood drawn at various intervals after the injection. With the assistance of Dr. J. H. McNeil, state veterinarian for New Jersey, 67 horses were each given subcutaneous injections of 5 cc of the 10 per cent. lamb brain suspension. The inoculations were made in a region where there were many cases of encephalomyelitis, and two of the inoculated animals developed the disease. The virus present in the one brain secured was highly virulent for guinea pigs and was evidently not the strain injected. The other 65 horses showed no reaction to the virus, except that many of those tested as well as all those inoculated at the laboratory developed neutralizing antibodies.

Testing the immunity of horses is a difficult problem because the only certain method of producing disease in these animals is by the intracerebral injection of virus and only a horse with a very high degree of immunity can withstand such an inoculation. Four out of nine vaccinated animals tested by this method showed no temperature reaction or other sign of infection. The other five animals, after an incubation period that was from one to two days longer than that in the controls, developed the disease and died. Two other vaccinated horses inoculated intravenously with virus showed no evidence of disease, but since only one of two controls was infected the results are not conclusive.

In spite of the fact that more than half of our vaccinated horses died from a test intracerebral inoculation, we believe that vaccination with the modified virus will protect against the natural disease. This belief is based on the results of the experiments with guinea pigs and on the facts that vaccinated horses developed neutralizing antibodies and that four horses became so highly immunized that they resisted the intracerebral injection of active virus.

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