times that needed to denature egg albumin, using the estimate given by Mirsky[12] or fifty times the energy needed for the inactivation of certain ionic forms of pepsin according to Steinhardt's determinations.[13] The denaturation of egg albumin by ultra-violet light is a first order reaction,[14] i.e., the survival curve is a straight line similar to that obtained for chromosome abnormalities. It seems quite likely, therefore, that denaturation may occur in the production of chromosome abnormalities by x-rays. In this connection it is interesting to note that abnormally high temperature will produce chromosome abnormalities. Also, with incompletely ripened *V. faba* seed that have thin seed coats, the frequency of chromosome abnormalities will gradually rise from 1 to 30 per cent in two months, suggesting that the process may be due to desiccation or oxidation. All these agents will denature proteins.

THE ACTION OF CERTAIN SUBSTITUTED PHENOLS ON MARINE EGGS IN RELATION TO THEIR DISSOCIATION

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It has been shown by Clowes and Krahl[1,2] that various substituted phenols as well as dinitrophenol increase the respiratory rate of marine eggs. Also, the highly interesting reversible block to cleavage, which they found to occur at the maximum of respiratory stimulation, is likewise exhibited. The different substances (nitro- and halo-phenols and cresols in particular) used were found to be active in different concentrations, and some attempt is made to relate the activity to molecular structure. The degree of dissociation of the phenolic OH is taken to be of no significance in their experiments. There has been some controversy concerning this question. Field, Martin and Field[3,4] showed that in yeast the amount of respiratory stimulation by 2,4-dinitrophenol and by 4,6-dinitrocresol depends upon the concentration of the undissociated form present, similar calculated concentrations of undissociated DNP giving at different pH's the same stimulation. Citing their own experiments and those of Ehrenfest and Ronzoni[5] on yeast, De Meio and Barron,[6] on the other hand, disagree with this conclusion.

Our experiments with sea-urchin eggs bear out the contention of Field, Martin and Field. In addition, experiments with different substituted phenols make it appear likely that once inside the cell, it is the dissociated form that is active.

The significance of the undissociated form became evident in experiments in which the effect of 2,4-DNP on different concentrations of eggs

was tried. A given concentration of DNP was found to be more effective in the higher concentrations of egg suspensions.[12] For example, in an experiment with the sea-urchin *Strongylocentrotus purpuratus*, it was found that a concentration of 3.75×10^{-5} molar DNP in sea water will permit 95% of the eggs of a weak suspension to divide, but will reversibly block cleavage in a heavy suspension, as table 1 shows. The result can be interpreted simply to be due to the lowering of pH caused by the greater CO_2 production of the eggs in the heavy suspension. Other experiments in which weakly buffered solutions were used and in which the pH was taken showed it to be, in fact, lower in the more concentrated suspensions (table 1, columns 4 to 7).

TABLE 1

EFFECT OF DIFFERENT CONCENTRATIONS OF EGGS. *Strongylocentrotus purpuratus*. TEMPERATURE, 20°C.

DNP CONCENTRATION $M \times 10^5$	EGG CONCENTRATION NO. PER CC. OF SOLUTION $\times$ 20,000	PER CENT CLEAVED	DNP CONCENTRATION $M \times 10^6$	RELATIVE CONCENTRATION OF EGGS	PER CENT CLEAVED	pH
2.25	1	100	2.0	1	98	7.25
2.25	25	40	2.0	115	73	7.19
3.75	1	95	3.0	1	60	7.24
3.75	25	0.5	3.0	115	15	7.13
5.25	1	60	4.5	1	40	7.21
5.25	25	0	4.5	115	5	7.09
0	25	100				

Such a pH effect can be attributed to a change in the concentration of undissociated DNP. To demonstrate this, it is necessary to show that at different pH's, the same effect is produced by the same calculated concentration of undissociated DNP. The results presented in table 2 show this relation.

In these experiments, the total carbonate components of the sea water were removed by acidifying to pH 3 and bubbling air through for 24 hours or more in order to eliminate CO_2 effects. It is necessary, then, to use another buffer in place of the bicarbonate system. For this purpose the dipeptide glycylglycine, as has been recently shown by us,[7] is a quite satisfactory buffering agent. Phosphate buffer is good only at low pH's; it begins to precipitate the Ca and Mg of sea water at pH 6.3. For dilute suspensions of eggs a 0.005 molar solution of glycylglycine in sea water gave sufficient buffering. For heavier suspensions, as used in respiration runs, concentrations of 0.01 to 0.02 molar were employed. No particular effect of the glycylglycine on the condition of the eggs is noted until concentrations of 0.05 to 0.10 molar are reached.

Table 2 gives the different concentrations of DNP required at different pH's to produce the same extent of reversible cleavage block. In de-

termining the blocking concentrations, a series of different concentrations were, of course, tested at each pH. The concentration of undissociated DNP present is calculated from the equation: $[HP]=\frac{[H^+][\Sigma P]}{[H^+]+K}$, where $[\Sigma P] = [HP] + [P^-]$, and K, the dissociation constant, is taken as 10^{-4}. It may be readily seen from the table that widely different concentrations of DNP are required at different pH's to give the same extent of blocking. However, the calculated concentrations of undissociated DNP present are practically constant at the different pH's. There is some divergence which may be significant because slightly lower values are consistently obtained at the lower pH's. We shall not try to account for this divergence at present. It is clear enough from the results that the effectiveness of DNP depends on the concentration of the undissociated form present.

TABLE 2

CONCENTRATIONS OF 2,4-DNP, $[\Sigma P]$, REQUIRED TO GIVE 90 TO 100% REVERSIBLE CLEAVAGE BLOCK AT DIFFERENT pH'S. $[HP]$ = CALCULATED CONCENTRATIONS OF THE UNDISSOCIATED FORM. *Strongylocentrotus purpuratus*. TEMPERATURE, 20°C.

EXPERIMENT	$[\Sigma P]$ $M \times 10^6$	pH	$[HP]$ $M \times 10^9$	EXPERIMENT	$[\Sigma P]$ $M \times 10^6$	pH	$[HP]$ $M \times 10^9$
	10.0	7.17	6.8		29.4	7.55	8.3
A	58.2	7.71	11.4	C	58.5	7.75	10.4
	10.0	7.00	10.0				
					12.0	7.22	7.2
	8.75	6.97	9.4		88.0	7.92	10.6
B	62.5	7.80	9.9	D	12.0	7.30	6.0
	8.75	6.99	9.2		88.0	7.92	10.6
	75.0	7.83	11.1		15.0	7.30	7.5

Similar results are obtained in respiration experiments with different concentrations of DNP at different pH's. For example, a 37.5×10^{-6} molar DNP solution at pH 7.70, increases the respiration to 201 per cent of the control rate, and an 8.0×10^{-6} molar solution is required to give a similar rise (240 per cent) at pH 7.22. The concentrations of undissociated DNP are 7.5×10^{-9} and 4.8×10^{-9} molar, respectively. Other substituted phenols that were tried showed the same pH effect.

It is evident, then, that comparisons of the activities of different substituted phenols must be based upon the concentrations of the undissociated form. A fairly exact knowledge of the dissociation constants is, of course, important. We have investigated the various phenols listed in table 3.

In this table, the concentrations of the undissociated form required to produce the same effect—90% reversible cleavage block—are given in the column marked $[HP]$. The dissociation indices used in calculating these concentrations are shown in the first column. A comparison of these con-

centrations shows enormous differences. The concentration of *o*-nitrophenol required is 1,000,000 times that of picric acid. Even omitting these extremes, we still have 2,6-dinitrophenol with an effectiveness 45,000 times that of *m*-nitrophenol. In other words, one molecule of 2,6-DNP is as effective as 45,000 molecules of *m*-nitrophenol. The effectiveness of the various substituted phenols might be assumed to depend on some structural property of the molecules. But none of the properties which we have studied or which have been suggested to us by structural chemists vary in the direction or in the relative magnitudes that would be necessary to account for these differences.

Further considerations, however, show that it may be unnecessary to explain these differences, or at least differences of such magnitude. If we assume that, once inside the cell, it is the dissociated rather than the undissociated form that is active, then the differences between the various

TABLE 3

CONCENTRATIONS, $[\Sigma P]$, OF VARIOUS SUBSTITUTED PHENOLS REQUIRED TO GIVE 90 TO 100% REVERSIBLE CLEAVAGE BLOCK AT pH 8.0. $[HP]$ = CALCULATED CONCENTRATION OF UNDISSOCIATED FORM; $[P^-]$ = CALCULATED CONCENTRATION OF THE ANION INSIDE THE CELL (INTERNAL pH 6.6). *Strongylocentrotus purpuratus*. TEMPERATURE 20°C.

	pK	$[\Sigma P]$ $M \times 10^5$	$[HP]$ $M \times 10^9$	$[P^-]$ $M \times 10^6$
o-nitrophenol	7.25[10]	364.0	550,000	122
m-nitrophenol	8.337[10]	34.3	240,000	4.4
p-nitrophenol	7.215[10]	14.9	21,000	5.1
2,4-dinitrophenol	4.00[10,11]	10.0	10	4.0
2,6-dinitrophenol	3.585[10]	13.9	5.3	5.5
2,4,6-trinitrophenol	0.796[11]	911.0	0.57	365
2,4-dichlorophenol	7.699[10]	30.9	103,000	8.2
2,4,6-trichlorophenol	6.0[10]	13.8	1,370	5.5

phenols largely disappear. For this purpose we must assume some value for the pH inside the cell. It is not necessary for purposes of comparison to know the exact pH inside the cell. There is now, however, fairly good agreement on the value 6.6, found by the Needhams[8] and by Chambers and Pollack,[9] for the pH of the sea-urchin egg. Using this value for the pH and the pK values given in column one of table 3 we get the calculated concentrations of the ions of the various substituted phenols given in the last column of the table. These values are for the most part quite close. Similar correspondence in $[P^-]$ is obtained in the respiration experiments For example, the concentrations causing maximum respiratory stimulation give the following calculated values for $[P^-]$ inside the cell: 2,4-DNP, 2.7×10^{-6} molar; *m*-nitrophenol, 3.4×10^{-6} molar; *p*-nitrophenol, 3.5×10^{-6} molar; 2,4-dichlorophenol, 6.2×10^{-6} molar. These values are obtained from respiration runs involving at least six different concentra-

tions. The values are in fairly good agreement. It is, of course, more difficult to determine the point of maximum respiratory stimulation than it is to determine cleavage block. We therefore make our comparisons on the basis of the same extent of reversible cleavage block caused by the different substituted phenols.

The values for *o*-nitrophenol and picric acid differ considerably from the others. *o*-Nitrophenol and picric acid also stimulate the respiration, giving, at the maximum, 215 and 125% of the control rate, respectively. The calculated $[P^-]$ at which this stimulation is obtained are 1.26×10^{-4} and 4.4×10^{-4}, respectively, which again departs from the others. Picric acid, because of its very high dissociation, might be expected to behave differently. Why *o*-nitrophenol should depart so much from the others cannot be so readily seen unless the listed dissociation constant is possibly too low. The agreement between the other nitrophenols is striking. The trichlorophenol also agrees, but the dichlorophenol value differs somewhat more from the others. For trichlorophenol another published value of its pK is 7.59[11] which would bring the calculated $[P^-]$ down to 2.6×10^{-6}. It would be well to know which pK value to take, and to know some of the others more accurately before attributing any significance to the differences. A biological method of determining dissociation constants from the analysis of the pH effects suggests itself for cases in which solubility or some such factor limits the application of physico-chemical methods. This method involves simply the determination of the concentrations of the particular substance required at different pH's to produce the same effect on the eggs. The method is limited to pK values lying in the pH range in which the eggs will develop.

The agreement between the $[P^-]$ inside the cell may be taken to mean that the various substituted phenols (with the exceptions noted) are equally effective. The experiments showing that the effectiveness of any one of these different substituted phenols is proportional to the concentration of the undissociated form present simply mean that it is the undissociated molecule that goes through the cell membrane. Once inside the cell it dissociates and it is apparently the ionic form that is active in stimulating respiration and reversibly blocking cleavage.

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[3] Field, J., 2nd, A. W. Martin, and S. M. Field, *Jour. Cell. and Comp. Physiol.*, **4**, 405–420 (1934).

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[6] De Meio, R. H., and E. S. G. Barron, *Ibid.*, **32**, 36–39 (1934).

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[9] Chambers, R., and H. Pollack, *Jour. Gen. Physiol.*, **10**, 739–755 (1927).

[10] From the *International Critical Tables of Numerical Data, Physics, Chemistry and Technology;* prepared by the National Research Council of the U. S. A., VI (1929).

[11] From Scudder, H., *The Electrical Conductivity and Ionization Constants of Organic Compounds.* New York, D. Van Nostrand and Company (1914).

[12] This has been noted by Krahl, Clowes and Taylor, *Biol. Bull.*, **71**, 400–400 (1936).

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*THE PRODUCTION OF STERILITY IN MALE MICE BY IRRADIATION WITH NEUTRONS**

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Recent investigations by Lawrence and Lawrence;[1] Lawrence, Aebersold and Lawrence;[2] Zirkle and Aebersold;[3] and Zirkle, Aebersold and Dempster[4] have shown that neutron rays act in a manner similar to, but not identical with, x-rays, in retarding or inhibiting the growth of various tissues. A striking difference is the much greater biological potency of neutron rays when measured in terms of ionization. There is evidence, moreover, that the difference between the two types of radiation varies according to the organism or tissue to which they are applied. Thus Zirkle, Aebersold and Dempster,[4] in comparing the biological activity of neutron and x-irradiation, find a ratio of effectiveness of 5 to 1 for actively sprouting seedlings, 2.1 to 1 for *Drosophila* eggs and 2.5 to 1 for fern spores. The results of Lawrence, Aebersold and Lawrence[2] suggest that fast neutrons are particularly effective in the case of cancerous tissue.

An investigation by Whiting[5] has shown that dominant lethal changes are produced in the sperm of *Habrobracon* by neutron rays.

The present study is concerned with the production of sterility and dominant lethal changes in mice by neutron irradiation.

Twelve mice of an inbred albino (*C*) strain were sent by air express from the Roscoe B. Jackson Memorial Laboratory to the Radiation Laboratory of the University of California for treatment. The arrangement for irradiation and dosage measurement is the same as that used by Zirkle, Aebersold and Dempster.[4] The dosages applied ranged, in the region of the testes, from 110 to 215 "roentgens" of neutrons. At the centers of the animals' bodies, which were nearer the source of the rays, doses ranged from 135 to 260 "r" of neutrons. Immediately following treatment, the mice were returned by air express, and placed on their arrival. each with two