25 of the patients treated. Seven additional patients were treated but could not be followed up.

Treponema were not found in the lesions of the first four cases 22–26 hr after treatment was started. The treponemal disappearance time in the next four patients was 23 hr. Two cases given 30 mg/kg/day were negative in darkfield microscopic examination in 26 hr.

Since there was rapid disappearance of the treponemas from the local lesions on a relatively large dose it was decided to investigate the minimal effective dose of the antibiotic. A single oral dose of 250 mg was given to three cases, the lesions remained positive for treponema in darkfield examination even at 48 hr. Two cases were given 500 mg in a single oral dose; the lesions in one became negative in darkfield examination in 48 hr, and the other remained positive even at 96 hr. In these cases treatment was subsequently instituted on a 30 mg/kg/day schedule for 6 days with complete healing of the lesions.

In order to determine whether the treponemal disappearance time could be decreased, one case was given 2 g every 4 hr for six doses, a second case 2.5 g every 4 hr for six doses, and a third case 12 g as a single dose; the trepomenal disappearance time was 24, 26, and 24 hr, respectively. Apparently increasing the dose of chloromycetin does not appreciably shorten the treponemal disappearance time.

These findings suggested that an oral chloromycetin dosage of 30 mg/kg/day divided into six doses and given at 4-hr intervals might be effective in the treatment of early syphilis. Therefore, a total of 24 patients were treated with 30 mg/kg/day for 4, 6, or 8 days. The lesions in all cases showed evidence of initial healing within 24 hr and most of them showed complete healing by the end of therapy. Some delay in healing occurred in a few cases where the location of the lesion predisposed to a slower response. For example, a patient with a urethral chancre took 6 days to heal because of the constant flow of urine over the lesion.

Chloromycetin seems to us to promote healing by a different mechanism from penicillin. Penicillin produces initial healing at the periphery of a lesion, whereas healing with chloromycetin therapy seems to be initiated from the base of the lesion. This is particularly striking in the large ulcers of benign late syphilis of the skin.

Quantitative serologic tests using the Eagle Flocculation Test for syphilis were performed prior to therapy and at monthly intervals after treatment. It is noted in the table that there is a rapid decline in serologic titer in most cases at the end of one month following treatment.

Two of the patients who received 30 mg/kg/day were pregnant. The clinical and serological response until now has been satisfactory in both patients. One patient has delivered a live child. Although serologically positive at birth, clinical and serologic progress in this child have been excellent without further therapy. The second patient is still pregnant.

The only toxic or untoward reactions noticed with the dosages of chloromycetin utilized in this study were an occasional mild diarrhea and an occasional complaint of dryness of the mouth. The Jarisch-Herxheimer reaction in chloromycetin-treated patients is either less frequent than that observed in penicillin-treated cases or of such a mild nature that it is frequently missed. Only two patients were observed to have very mild reactions 48 hr after treatment was started. Several patients complained of a generalized aching sensation 48 hr after treatment began, but no fever or eruptions were noted. As these symptoms disappeared in 8–12 hr, despite further therapy, they may have been Jarisch-Herxheimer reactions.

Since relatively small doses of chloromycetin will cure acute gonorrhea, (*2, 3*) attention should be called to the possible danger of masking the diagnosis of syphilis.

Studies with the use of chloromycetin for the treatment of syphilis are continuing, and are being extended to include the late manifestations of syphilis. One patient with late syphilis manifested by extensive gummata of the leg is now under therapy with chloromycetin. This patient has had a dramatic healing response within 4 days after treatment was started.

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Effect of Pressure on Induction of Mutations by Nitrogen Mustard[1]

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An analysis of the mechanism of action of mutagenic agents depends, for the most part, upon indirect experimental methods (*1*). By studying changes in the mutation rate under varying experimental conditions, such as temperature and pH, it has been possible to obtain some idea as to the general stability of genes. Since nucleoproteins are apparently the most important components of the chromosome (*5*) (and presumably of the genes), it is likely that structural changes in these molecules underlie the basic alterations involved in gene mutation. Such structural changes might be of the order of magnitude observed by Johnson, Eyring, and collaborators in protein denaturation (*14*). Since pressure has been used successfully in the analysis of such equilibria or rate processes by these workers, it seemed to the authors that a similar study of the effect of pressure on the mutagenic action of the nitrogen mustards would aid materially in interpreting the results which have been obtained with these and similar agents. According to the general expressions which have been derived for a quantitative interpretation of the effect of pressure on biological processes (*8*), it should be possible to determine the magnitude of the volume change which occurs during the reaction that results in a gene mutation.

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A series of experiments have been performed to determine the effect of high hydrostatic pressure on the induction of both morphological and biochemical mutants in *Neurospora crassa* by nitrogen mustard. The results, summarized in Table 1, clearly show that application of high pressure to a conidial suspension containing nitrogen mustard depresses the number of morphological mutants obtained ($P < .001$). The reduction of the mutation rate is directly proportional to the pressure applied. With 9,000 lb per sq in. (psi), the rate of morphological mutation is reduced approximately 45–50%, while with 5,000 psi the rate is reduced approximately 30–35%. A few experiments performed at a pressure of 2,000 psi indicate that approximately 10% reduction occurs at that pressure. A record of the different types of morphological mutants which occurred was kept, and the results indicate that no particular sort of mutant was suppressed by the high pressure. The mutants recovered after subjection to mustard plus 9,000 psi were of the same general types as those recovered after treatment with mustard at normal atmospheric pressure. Results obtained with different pressures may be compared, therefore, on a rate basis.

By making use of the following general equation, the volume change of a reaction can be determined by plotting the *ln* of the velocity constant against pressure:

$$\left(\frac{d \ln K}{dp}\right)_T = \frac{\Delta V}{RT}$$

From a plot of the *ln* of the morphological mutation rate against pressure, the present data indicate that an average volume change for the individual event occurring in the chromosome is approximately 70 cm^3 per mole, a volume change which is similar to that found for denaturation of serum globulin (*11*), for inactivation of specific antitoxin (*12, 13*), and for specific precipitation (*4*).

Pressure appears to have an opposite effect to that just described when, instead of morphological mutants, chemical mutants induced by nitrogen mustard are tabulated. In all experiments there is a consistent increase in the number of biochemical mutants obtained when pressure is combined with nitrogen mustard. On the basis of kinetic analysis this relationship indicates that changes which result in the formation of a biochemical mutant proceed with a decrease in volume. Additional experiments will be necessary to establish this point definitely.

The differential effect of pressure on the induction of morphological and biochemical mutants by nitrogen mustard is of particular interest because it confirms previous indications that these two types of mutants are different in nature. Experiments combining the effects of ultraviolet radiation and nitrogen mustard have shown that a difference between these two types of mutants exists (*15*). A large percentage of the morphological mutants are sterile, a fact indicating that probably the phenotypic effect is due to gross chromosomal changes; whereas biochemical mutants, on the other hand, usually require single growth factors and have been shown, in many cases, to be due to single gene changes (*3*). It is not surprising that pressure has such a marked effect on the rate of morphological mutations if most of them are due to chromosomal breaks. Such rearrangements of the nucleoproteins would be expected to be accompanied by large volume changes. It should be emphasized that although our results show a significant decrease in the number of morphological mutants at high pressure, the percentage of viable spores is not significantly altered. This indicates that the killing action of the mustard is not inhibited by the pressure.

The possibility that cross-linking plays a role in the action of mustard gas has been suggested (*6, 10*). Elmore *et al.* have found that the number of blocked titratable groups in thymus nucleic acid is greater than the number of mustard gas residues in the molecule. Their results indicate that the mustard gas molecule reacts with two titratable groups either in the same molecule or, by cross-linkage, between different polynucleotide chains. The increased viscosity they observed in the treated sample also suggested the existence of intermolecular cross-linkages. Goldacre *et al.* further suggested that nitrogen mustards produce their effects by interchromatid cross-linkages, which result in fragmentation and consequently in rearrangement. It seems likely that such extensive rearrangements of the nucleoproteins in the chromosome as that which results after treatment with nitrogen mustards may be the basis of the morphological effect observed in subsequent generations. Furthermore, it is to be expected that these particular reactions would be accompanied by large volume changes and that pressure would influence the process significantly.

The effect of pressure on the process yielding biochemical mutations indicates that such an extensive rearrangement of genic material does not occur in this case. The evidence suggests, on the contrary, that such mutations are accompanied by a volume decrease, such as might be expected to occur with a simple bimolecular reaction (*7*). A more extensive analysis of the effect of pressure to include not only single gene mutations but also small deletions involving several genes must be of great interest. It may be that changes which result in a single gene mutation are greatly delayed and occur only at the time of the reduplication process. Combination of nitrogen mustard with groups at the surface of the chromosome or gene may perhaps take place with a small volume change; however, if the genes have to unfold in the reproduction process, as has been suggested (*9*), it is possible that exposure of new groups at that time to the attached nitrogen mustard molecule might greatly influence the unfolding process. Analysis of delayed effects of pressure should throw light on the nature of this process.

Experiments have been performed to determine whether pressure would have an effect if applied after mustard treatment. In these experiments treated spores were centrifuged and resuspended in phosphate buffer and immediately placed under pressure. If 9,000 psi are applied within 10–15 min after the treated spores have been resuspended in phosphate buffer, it is possible to reduce the number of morphological mutants by approximately 25%, in contrast to the 45–50% reduction observed when pressure is applied simultaneously with mustard. Thirty min after treatment the mutation process appears to have become irreversible insofar as morphological mutants are

TABLE 1

EFFECT OF PRESSURE ON INDUCTION OF MUTATIONS BY NITROGEN MUSTARD IN *Neurospora crassa* (TEMP = 26° C)

Exp. No.	Treatment	No. isolations	No. morph. mutants	% Mut.	No. biochem. mutants	% Mut.	% Kill
21	0.2% Mustard	357	53	14.9	3	0.8	99.57
	0.2% Mustard + 9000 psi	238	17	7.2	6	2.5	99.94
25	0.2% Mustard	396	45	11.4	4	1.0	99.29
	0.2% Mustard + 9000 psi	435	33	7.6	8	1.8	99.72
48	0.2% Mustard	423	82	19.4	8	1.9	....
	0.2% Mustard + 9000 psi	377	46	12.0	10	2.7	....
53	0.2% Mustard	329	49	15.0	..	..	....
	0.2% Mustard + 9000 psi	595	44	7.4	..	..	....
29	0.2% Mustard	323	27	8.3	..	..	98.27
	0.2% Mustard + 5000 psi	377	20	5.3	..	..	98.50
39	0.2% Mustard	513	63	12.3	4	0.8	....
	0.2% Mustard + 5000 psi	448	36	8.0	5	1.1	....
	None	400	5	1.4	0	0	....
	9000 psi	385	3	0.8	0	0	50.00

concerned, for pressure applied at that time has no appreciable effect. The results therefore indicate that there are temporary delayed effects of nitrogen mustard which can be influenced by application of high hydrostatic pressure.

From the type of temperature and pressure analysis which has been adequately outlined by Johnson, Eyring, and their co-workers, it should be possible to obtain a clearer understanding of the action of chemical agents and various types of radiation in inducing genic changes. The action of pressure on the combined effects of various mutagenic and nonmutagenic agents (N-mustard, x-ray, ultraviolet, and infrared radiation, etc.) may be expected to clarify greatly the process by which these environmental factors can modify the mutation process.

A microconidial strain of *Neurospora crassa*[2] (*2*) was used to study the effect of pressure on the induction of both biochemical and morphological mutants by nitrogen mustard, bis-β-chloroethylmethylamine. Eight-day-old conidia were suspended in 0.1 M phosphate buffer (pH 6.5) and filtered through a sterile pad of cotton in order to remove any mycelial fragments. After the desired treatment, the spores were plated onto a complete medium containing 1.5% L-sorbose (*16*). After 2–3 days, single isolates were transferred to small (10 × 75 mm) complete slants and subsequently scored for morphological mutations. These isolates were then transferred to a liquid minimal medium to determine the presence of any biochemical mutants. Most such mutants found involved deficiencies of single amino acids, vitamins, or purines.

The method used in applying high pressure to the conidial suspension was essentially that which has been described by Johnson *et al.* (*14*). The desired amount of an aqueous solution of nitrogen mustard was mixed with the conidial suspension, and a sample of the mixture was placed in the high pressure bomb. In those cases where pressure was applied simultaneously with the mustard, there was a lapse in time of approximately 30 sec between mixing of the mustard with the conidial suspension and application of the pressure. After treatment under high pressure, a sample of the mixed suspension was removed from the pressure bomb and rapidly plated onto the complete medium.

[2] Kindly supplied by Dr. E. L. Tatum.

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A Prediction Regarding the Humboldt Current

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According to a finding of the late E. G. Mears, an unusual ice outbreak was due in the North and South Atlantic during the proper seasons of 1949. The Humboldt Current should begin receiving warm water invasions by the southern summer of 1950, but these invasions should not become serious until 1951–52. Dr. Mears' attention was drawn to the Humboldt Current during the 1941 conference of scientists in Lima. In *Science* (*16*) of April 24, 1942, he pointed out that the 1941 tropical intrusion (*9*) into the normally subtropical Peru, did not correspond to the seven-year cycle (*24*), which had been proposed previously, for only two years had elapsed since the 1939 interruption had occurred. In Dr. Mears' subsequent study of the Humboldt Current, he became con-

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