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1. Hamdy, M. K., Deatherage, F. E., Shinowara, G. Y., Proc. Soc. Exp. Biol. and Med., 1957, v95, 255.
2. Hamdy, M. K., May, K. N., Powers, J. J., ibid., 1961, v108, 185.
3. Hamdy, M. K., May, K. N., Powers, J. J., Pratt, D. E., ibid., 1961, v108, 189.
4. Hamdy, M. K., Kunkle, L. E., Deatherage, F. E., J. Animal Sci., 1957, v16, 490.
5. Hamdy, M. K., Rheins, M. S., Deatherage, F. E., Proc. Soc. Exp. Biol. and Med., 1958, v98, 763.
6. Hamdy, M. K., Kunkle, L. E., Rheins, M. S., Deatherage, F. E., J. Animal Sci., 1957, v16, 496.
7. Hamdy, M. K., May, K. N., Powers, J. J., Poultry Sci., 1961, v45, 790.
8. Merezhinskii, M. F., Yadvinskaya, E. S., Ivanova, V. S., Ukrain Biokhim. Zhur., 1954, v26, 435.
9. Gjessing, E. C., Ludewig, S., Chanutin, A., J. Biol. Chem., 1947, v170, 551.
10. White, Betty N., Shetlar, M. R., Shurley, Helen M., Schilling, J. A., Proc. Soc. Exp. Biol. and Med., 1959, v101, 353.
11. Guschlbauer, W., Williamson, M. B., Can. J. Biochem. and Physiol., 1963, v41, 820.
12. Engel, F. L., Ann. N. Y. Acad. Sci., 1952, v55, 381.
13. Reynolds, B. L., Leveque, T. F., Buxton, R. W., Am. Surg., 1963, v29, 48.
14. Schmittle, S. C., Rogers, A. N., 1964, unpublished data.
15. deDuve, C., Lyosomes, a new group of cytoplasmic particles. In: Cytoplasmic Particles, Teru Hayaski, Ed., Ronald Press, N. Y., 1959 pp. 128-159.
16. Brown, W. E., Hamdy, M. K., J. Food Sci., 1964, v29, 407.
17. Schneider, W. C., Hogeboom, G. H., J. Biol. Chem., 1952, v198, 155.
18. Fishman, W. H., Springer, E., Brunetti, R., ibid., 1948, v173, 449.
19. Sellinger, O. Z., Beaufay, H., Jacques, P., Doyen, A., deDuve, C., Biochem. J., 1960, v74, 450.
20. Gornall, A. G., Bardawill, C. T., David, M. M., J. Biol. Chem., 1949, v177, 751.
21. Wattiaux, R., deDuve, C., Biochem. J., 1956, v63, 606.
22. Carranza, F. A., Jr., Cabrini, R. L., Science, 1962, v135, 672.
23. ———, J. Invest. Dermatol., 1963, v40, 27.
24. Brody, S., Nature, 1958, v182, 1386.
25. Daoust, R., Cantero, A., J. Histochem. Cytochem., 1959, v7, 139.
26. Daoust, R., Haruko, A., Cancer Res., 1963, v23, 131.
27. Ellem, K. A., Cotter, J. S., Kuhn, J., Nature, 1959, v184, Suppl. 13, 984.
28. deDuve, C., Scient. Am., 1963, v208, 64.
29. Zalkin, H., Tappel, A. L., Caldwell, K. A., Shibko, S., Desai, I. D., Holliday, T. A., J. Biol. Chem., 1962, v237, 2678.
30. Tappel, A. L., Zalkin, H., Caldwell, K. A., Desai, I. D., Shibko, S., Arch. Biochem. and Biophys., 1962, v96, 340.
31. Cohn, Z. A., Hirsch, J. G., J. Exp. Med., 1960, v112, 983.

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## Some Biological Changes in Traumatized Poultry Muscle.* (30300)

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Decreases in the adenosinetriphosphate (ATP) content of various tissues after different types of injury have been reported, usually accompanied by an increase in inorganic phosphate(1-4). Uyeki(3) reported that the predominant changes produced by X-irradiation were on the adenosinetriphosphate levels, and that the magnitude and the temporal sequence of this decrease varied from one species to another. Less significant changes were noted in the total adenosine nucleotide, adenylic acid, and adenosinediphosphate levels of spleen in rats, whereas in the mouse the adenylic acid levels increased 65 to 75% above the normal level 3 to 7 days following exposure to irradiation. Engel(1) and Jordan and Gray(4) found

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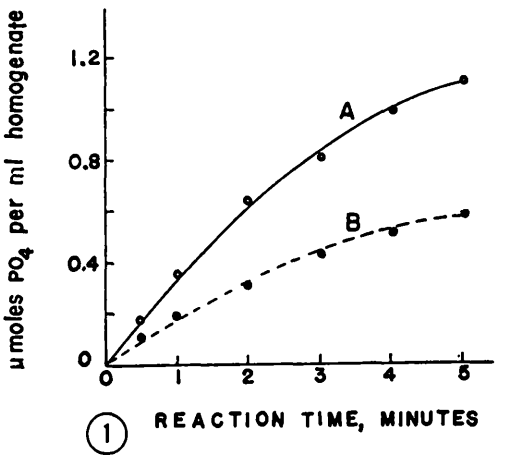
diminished ATP, ADP and creatine phosphate and increased inorganic phosphate, lactate and pyruvate following trauma. The latter authors established a significant drop in brain ATP content within 15 minutes of injury to that organ and the level remained low throughout the regenerative process. Pennington(5) reported that mitochondrial ATPase in muscle of dystrophic mice was 15% in excess of the normal level. DuBois and Petersen(6) noted increased ATPase and 5-nucleotidase activities in hematopoietic tissues obtained from irradiated rats. Studies on the ATPase content of tumors and tissues from tumor bearing mice(7) revealed high mitochondrial ATPase in the Galliera sarcoma, whereas the nucleotidase of the liver was depressed and no change was observed in kidney or skeletal muscle.

The present study was carried out to determine the effect of trauma on ATPase activity, as well as alterations in glycogen and lactic acid, in poultry muscle as a result of trauma to the muscle itself.

*Materials and methods. Bruising and sampling.* Experimental chickens were 8 to 10 weeks old, mixed-sex White Leghorns. They were maintained in 22°C constant temperature rooms and were offered standard rations and water *ad libitum.* Bruises were inflicted on the pectoralis major muscle using the standard technique of Hamdy *et al*(8). At various time intervals after bruise infliction, bruised and control chickens were sacrificed by decapitation, the tissues were immediately excised, wrapped in Cryovac packaging film, immersed in ice and used for analysis within 3 hours.

*Assay for ATPase.* Substrate was prepared to contain 2 mg of disodium adenosinetriphosphate (Nutritional Biochemicals Corp.) per ml of water and the pH was adjusted to 7.0 with 0.1 N NaOH. Reaction tubes contained 1.0 ml of substrate, 1.0 ml of trihydroxymethylaminomethane (Tris) buffer (pH 7.4, 0.2 M), and 1.0 ml of 2% tissue homogenate. (The 2% tissue homogenate, w/v, was prepared in cold distilled water using a Servall Omni-mixer.) Reactions were incubated for 5 minutes at 37°C and were then stopped by adding 7.0 ml of trichloroacetic acid. Following centrifugation, the inorganic phosphate in the enzyme-substrate reaction supernate and in substrate and tissue control tubes was determined by the method of Lowry and Lopez(9). Protein estimations were effected on homogenates using the alkaline copper reagent of Gornall *et al*(10) and the specific activity of the enzyme was expressed as the μmoles of inorganic phosphate liberated from ATP per mg tissue protein per 5 minutes incubation at 37°C. At no time did the activity of this enzyme appear strictly linear, although it approached linear proportions (Fig. 1). It is possible that the short period of linearity for the enzyme was due to the inhibitory effect of the ADP formed, since it is known that ADP completely inhibits myo-



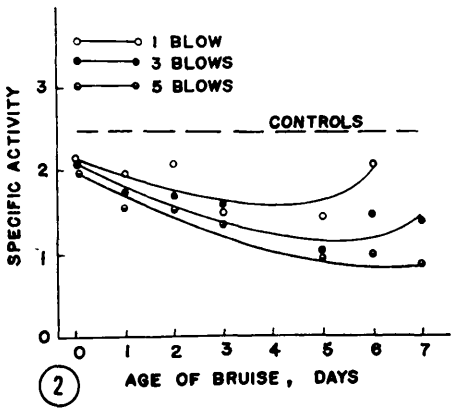




FIG. 1. ATPase reaction rates using 1.0 ml of a 4% tissue homogenate (A) and 1.0 ml of a 2% tissue homogenate (B).

FIG. 2. Effect of severity and age of bruise on myosin ATPase activity in poultry bruises.

TABLE I. Effect of Bruising and Age of Bruise on ATPase Specific Activity.*

| Age of bruise, days | Exp 1 | | Exp 2 | | |
|---|---|---|---|---|---|
| | No. of birds | Specific activity (no $Ca^{++}$) | No. of birds | Specific activity | |
| | | | | No $Ca^{++}$ | $3.3 \times 10^{-3}$ M $Ca^{++}$ |
| 0 | 4 | .951 ± .09 | 3 | .849 ± .03 | 1.447 ± .05 |
| 1 | 4 | .926 ± .07 | 3 | .799 ± .05 | 1.287 ± .09 |
| 2 | 4 | .878 ± .02 | 3 | .921 ± .10 | 1.605 ± .11 |
| 3 | 4 | .852 ± .02 | 3 | .737 ± .09 | 1.352 ± .12 |
| 4 | 4 | .830 ± .12 | 3 | .942 ± .08 | 1.680 ± .08 |
| 5 | 4 | .724 ± .48 | 3 | .685 ± .09 | 1.341 ± .15 |
| 6 | 4 | .744 ± .12 | 3 | .549 ± .07 | 1.252 ± .26 |
| 7 | 4 | .732 ± .10 | 3 | .775 ± .10 | 1.483 ± .21 |
| Control (no bruise) | 4 | 1.071 ± .07 | 3 | 1.002 ± .18 | 1.512 ± .37 |

* Expressed as μmoles of inorganic phosphate liberated from ATP per mg tissue protein per 5 min incubation at 37°C.

sin ATPase(11). ATPase activity in 2 and 20% tissue homogenates was completely destroyed after heating at 80°C for 10 min or by centrifugation at 3,000 × *g* for 5 minutes. Activity was stimulated *in vitro* by incorporation of $3.3 \times 10^{-3}$ M $CaCl_2$ in the reaction mixture.

*Assays for glycogen and lactic acid.* While chickens were under Nembutal anesthesia, tissue samples were collected with a sharpened no. 2 cork borer. Samples for lactic acid analysis(12) were placed in preweighed tubes containing 5.0 ml concentrated sulfuric acid; those for protein determinations(10) in preweighed tubes containing 3.0 ml distilled water; and those for glycogen assays were placed in preweighed tubes containing 3.0 ml of 30% KOH. Glycogen was precipitated according to Good *et al*(13) and was quantitatively determined by anthrone reagent using glucose as standard(14). The center of the bruise, its periphery and symmetrically located control muscle were analyzed.

*Results. Effect of age of bruise. ATPase.* In the first experiment, 32 bruised and 4 control chickens were used. ATPase was measured without $CaCl_2$. In the second experiment, 24 bruised chickens and 3 controls were employed and the enzyme activities were assayed with and without $3.3 \times 10^{-3}$ M $CaCl_2$. The results are shown in Table I. In both experiments, the enzyme assayed without calcium showed a gradual loss of activity commencing immediately after bruising and continuing through the latter stages of healing. In the first experiment the lowest activity (28% below control activities) occurred 5 days after trauma and in the second experiment, the lowest activity (45% below controls) was attained on the sixth day following injury. When assays were performed in the presence of $3.3 \times 10^{-3}$ M $CaCl_2$, the enzyme activity in all bruised tissue homogenates was within the range of the standard deviation of controls (1.51 ± 0.37).

*Effect of severity of bruising.* After the decrease in ATPase was noted as a result of trauma, it was decided to examine factors other than age of bruise which might affect the ATPase activity. Accordingly, 3 groups of 24 birds each were used. Birds of the first group were bruised using one blow, the second with 3 blows and the third with 5 blows. In addition, 4 unbruised chickens served as controls. The results are shown in Fig. 2. Mean specific activity immediately after bruising was 13 to 20% below the average control activity. Activity in all 3 groups (1, 3 and 5 blows) diminished gradually with time post contusion and appeared to return to the normal value upon complete healing as evidenced by gross appearance. As the severity of the bruise increased, the specific activity of the ATPase enzyme decreased. The lowest level of activity was reached on the fifth day with both the one-blow and 3-blow bruises and on the seventh day with 5-blow bruises. Increasing the severity of injury caused a marked loss in activity in bruises of all ages and, also, tended to prolong the time required to reach the lowest level of activity. Thus, the changes of ATPase activity were directly related to

TABLE II. Effect of Successive Bruising on ATPase Activities of Tissues.*

| No. of birds | Age of bruise | Specific activity | | |
|---|---|---|---|---|
| | | 1st bruise | 2nd bruise | 3rd bruise |
| 9 | 0 hr | .74 | .75 | .78 |
| 9 | 3 days | .73 | .67 | .62 |

* Control tissue = 1.071 ± .07.

the severity of contusion. Maximum loss of activity with one blow (47%) and with 3 blows (62%) occurred on the fifth day post injury. Maximum activity loss with 5 blows (77%) did not occur until the seventh day after bruise infliction.

*Effect of successive bruising.* Three groups of chickens were used in this experiment. The first group was bruised using 3 blows; some of these were sacrificed at 0 hr and others at 3 days. The bruised tissues were excised and assayed for ATPase activity. The second group was treated similarly except that each bird was bruised a second time 2 days later using the same applied force. The third group was bruised on 3 occasions 2 days apart. The results (Table II) indicate that immediately following bruise infliction, the ATPase activity decreased rapidly in the 3 groups as compared to the control group. Three days after trauma, the tissue of birds given 2 or 3 separate bruises exhibited lower ATPase activity compared to those receiving only one bruise.

*Effect of age of bruise on glycogen and lactic acid.* Glycogen concentration in bruised muscle fell to less than ⅓ of normal level within 12 hours of injury and at the end of 7 days was still only ½ of control levels (Table III). Glycogen in the bruise periphery was intermediate in concentration between the bruise itself and the symmetrically located muscle obtained from the unbruised breast of bruised chickens.

TABLE III. Glycogen Content in μg per mg Protein of Tissue at Bruise Center, Bruise Periphery and Symmetrically Located Control Muscle.

| Time after bruising | No. of birds | Center | Periphery | Control |
|---|---|---|---|---|
| 0 hr | 5 | 115 ± 35 | —* | 134 ± 32 |
| 12 " | 5 | 39 ± 7 | 83 ± 21 | 104 ± 27 |
| 1 day | 7 | 33 ± 15 | 58 ± 37 | 96 ± 40 |
| 2 days | 7 | 37 ± 21 | 73 ± 24 | 94 ± 25 |
| 7 " | 7 | 66 ± 56 | 76 ± 33 | 123 ± 25 |

* Samples were not analyzed.

Lactic acid concentrations measured in bruises, bruise peripheries and symmetrically located control muscles from bruised chickens at any given time post contusion were markedly similar (Table IV). At different time intervals following injury, however, there were striking differences. Lactic acid appeared to accumulate in the bruise immediately following bruise infliction. By 24 hours there was a marked drop in lactic acid levels and the concentration remained low throughout the next 7 days.

TABLE IV. Lactic Acid Content in mg per g of Dry Weight Tissue at Bruise Center, Bruise Periphery and Symmetrically Located Control Muscle.

| Time after bruising | No. of birds | Center | Periphery | Control |
|---|---|---|---|---|
| 0 hr | 5 | 167 ± 74 | —* | 110 ± 35 |
| 12 " | 5 | 172 ± 124 | 127 ± 34 | 149 ± 53 |
| 1 day | 5 | 37 ± 14 | 36 ± 6 | 34 ± 6 |
| 2 days | 5 | 32 ± 6 | 37 ± 9 | 36 ± 9 |
| 7 " | 5 | 65 ± 14 | 68 ± 10 | 70 ± 16 |

* Samples were not analyzed.

*Discussion. ATPase.* The response of ATPase to bruising was a reduction in activity which was significant when measured without calcium activation. Two possible explanations are offered to account for this decrease in activity. Hemoglobin and its degradation products, which are known to accumulate in bruised tissues(15), may sequester divalent ions ($Ca^{++}$ and $Mg^{++}$) and render them less available for enzymatic functions. It is also probable that accelerated proteolytic activity in bruised tissue as previously noted by Hamdy *et al*(16) affects the ATPase enzyme by a process of denaturation and/or destruction. These proteolytic enzymes are of lysosomal origin(22). The ATPase activity measured in traumatized tissue was predominantly that of myosin. This assumption is substantiated by the fact that the enzyme is water insoluble and its activity is enhanced by calcium ions. Calcium ($3.3 \times 10^{-3}$ M $CaCl_2$) stimulated ATPase activity in tissue homogenates from both normal and contused pectoral muscle, but it did not completely restore

the ATPase activity in the bruised areas to normal tissue levels. Increasing the severity of injury caused a marked loss of activity in bruises of all ages and, also, tended to prolong the time required to reach the lowest level of activity. Thus, the ATPase activity changes were directly related to the severity of contusion. Examination of the tissues of birds bruised 1, 2 or 3 successive times revealed that each successive bruise had a lower measurable ATPase activity at 3 days after trauma. These lowered activities suggested that the ATPase in the tissues of birds bruised 2 or 3 successive times reacted to this trauma as if these tissues had been severely damaged. These results correlated with those previously reported on the severity of the bruise. It is possible that the ATP changes (or nucleotide changes) reported here may reflect alterations in tissue morphology as indicated in our previous studies on acid and alkaline phosphatase activity of bruised tissue(17) and to the limitation in the infiltration of leucocytes and phagocytes.

*Glycogen and lactic acid.* The rapid disappearance of glycogen from the injured site (Table II) was in accord with the data of other investigators following various types of injury(18,19). Glycogen remained low in the necrotic bruise center but tended to be somewhat higher in less necrotic areas of injury (the periphery), indicating either less glycolysis or increased glycogen synthetase in the healing periphery than in the necrotic bruise. Whether the glycogen changes in the bruise reflected an increased anaerobic metabolism (anoxia) causing a loss of glycogen synthetase activity and/or other enzymes regulating glycogen deposition or an increased energy demand on the part of the injured area cannot be ascertained from available data.

It is interesting to note that the highest concentrations of lactic acid (Table III) found in bruised chickens coincided with the period of glycogen loss in the bruise. It is tempting to conclude that this lactic acid was formed from glycogen by glycolysis. Using the same line of reasoning, it seems logical to assume both a low rate of glycogen synthesis and glycogen breakdown in 2- through 7-day-old bruises. The lack of synthesis was indicated by the continued low level of glycogen in the bruise and the low rate of glycolysis was indicated by decreased lactic acid concentration.

To explain the similarity of the bruise, periphery and symmetrically located control muscle of individual chickens one must say, as suggested by Lilienthal and Zierler(20), that lactate diffuses from muscle into the blood very rapidly. Hence, blood and muscle lactate would be in a constant state of approximate equilibrium.

Shimizu and Hamuro(21) associated high alkaline phosphatase activity with glycogen deposition in brain stab wounds of mice. The present investigation failed to demonstrate glycogen accumulation or elevated alkaline phosphatase(17) in poultry bruises undergoing necrosis.

*Summary.* Experimentally inflicted poultry bruises were examined for ATPase, glycogen and lactic acid. ATPase activity decreased as a result of trauma and was dependent on age of bruise, severity of trauma and previous bruise history. Glycogen concentrations decreased within 12 hours of injury whereas lactic acid increased immediately following contusion. By 24 hours post trauma, both lactic acid and glycogen levels were considerably below normal levels and continued to be low throughout the healing process.

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1. Engel, F. L., Ann. N. Y. Acad. Sci., 1952, v55, 381.

2. Endo, S., Ono, Y., J. Med. Sci., 1958, v5, 141.

3. Uyeki, E. M., Radiation Res., 1959, v11, 617.

4. Jordan, W. J., Gray, I., J. Biol. Chem., 1955, v215, 669.

5. Pennington, R. J., Biochem. J., 1961, v80, 649.

6. DuBois, K. P., Petersen, D. F., Am. J. Physiol., 1954, v176, 282.

7. Nante, L., Tumori, 1961, v47, 221.

8. Hamdy, M. K., May, K. N., Powers, J. J., Poultry Sci., 1961, v45, 790.

9. Lowry, O. H., Lopez, J. A., J. Biol. Chem., 1946, v162, 421.

10. Gornall, A. G., Bardawell, C. T., David, M. M., ibid., 1949, v177, 751.

11. Green, I., Monmaerts, W. F. H. M., ibid., 1954, v210, 695.

12. Barker, S. B., Summerson, W. H., ibid., 1941, v138, 535.
13. Good, C. A., Kramer, H., Somogyi, M., ibid., 1933, v100, 485.
14. Seifter, S., Dayton, S., Novic, B., Muntwyler, E., Arch. Biochem., 1950, v25, 191.
15. Hamdy, M. K., Deatherage, F. E., Shinowara, G. Y., Proc. Soc. Exp. Biol. and Med., 1957, v95, 255.
16. Hamdy, M. K., May, K. N., Powers, J. J., ibid., 1961, v108, 185.
17. Brown, W. E., Hamdy, M. K., J. Food Sci., 1964, v29, No. 2, 407.
18. Butcher, E. O., Klingsberg, J., Proc. Soc. Exp. Biol. and Med., 1962, v111, 783.
19. Beatty, C. H., Am. J. Physiol., 1945, v143, 579.
20. Lilienthal, J. L., Zierler, K. L., Biochemical Disorders in Human Diseases, R. H. S. Thompson, E. J. King, Ed., Academic Press, Inc., New York, 1957, p445.
21. Shimizu, N., Hamuro, Y., Nature, 1958, v181, 781.
22. Desai, I. D., Calvert, C. C., Scott, M. L., Tappel, A. L., Proc. Soc. Exp. Biol. and Med., 1964, v115, 462.

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## Residual Effects of a Single Exposure to Hyperbaric Oxygen in Mice.* (30301)

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The use of hyperbaric oxygenation is gaining acceptance as therapy in a number of medical and surgical diseases(1). However, this therapy often requires several treatments for a number of days, and there is evidence that repeated exposures to high pressures of oxygen might sensitize the central nervous system to oxygen toxicity. Almeida reported in 1934 that rats exposed to oxygen at 6 atmospheres of pressure (absolute) until they convulsed, in subsequent exposures would convulse within ⅓ to ¼ the initial time(2). Bean reported similar results(3). Fenn observed that daily exposures of the fruit fly to 1 atm. of $O_2$ for 6 hours every day, will decrease the life span by 10 to 15%(4).

The present experiments were planned to determine if repeated exposure to hyperbaric $O_2$ in the range used clinically would sensitize mice to convulsions. The length of exposure used was selected so as not to produce any convulsions during the first pressurization.

*Method.* Male Paris mice weighing between 25 to 30 g were exposed to hyperbaric oxygen in a small, transparent, lucite pressure chamber. The floor of the chamber was covered with a carbon dioxide absorbant (baralyme), and before pressurization the chamber was flushed with 100% $O_2$ for 5 minutes and then brought to the desired pressure in 4 to 5 minutes. Gas flows were adjusted to maintain a constant pressure in the chamber while allowing some $O_2$ to continuously escape. The mice were constantly observed throughout pressurization and the time of onset of convulsions was recorded. The animals were subjected to a varied number of exposures at either 3 atmospheres pressure absolute for 15 minutes or 4 atm. press. absolute for 5 minutes. When more than one exposure per day was given, an hour was allowed to elapse between experiments. Between exposures the animals were given food and water *ad lib.*

In the first series of experiments, 144 mice were exposed to 3 atm. press. for 5 days: 48 mice were exposed once a day; 24 twice a day; 24 four times a day and 48 once every other day (3 exposures). In the second series, 288 mice divided into 6 groups were exposed to 3 atm. press. and during the following 6 days, one of the groups was given an additional exposure. The delay between exposures thus varied from one to 6 days. In the third series of experiments, 144 mice were exposed to 4 atm. press. for 5 days: 24 mice were exposed once a day; 48 twice a day; 24 four

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