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Neutralizing formaldehyde in chicken cadaver with urea and urea fertilizer solution

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ABSTRACT. This study demonstrated the potential of using urea and urea fertilizer to neutralize formaldehyde (Fd) in chicken cadavers. Initially, *in vitro* Fd neutralization with various concentrations of urea solution (US) and urea fertilizer solution (UFS) was conducted; subsequently, 18% US and 27% UFS were selected for infusing into the formalinized chickens. The measurement at 48 hr after infusion showed that both solutions could effectively lower Fd in chicken cadavers to below a permissible exposure limit without affecting cadaveric and histological quality. In addition, neutralizing power of 18% US was approximately 1.3 times that of 27% UFS. This is the first demonstration of neutralizing potential of US and UFS against Fd both *in vitro* and *in vivo*.

KEY WORDS: chicken cadaver, formaldehyde, neutralization, urea, urea fertilizer

Formaldehyde solution (FS) has been widely used as a principle component of embalming fluid for preparing cadavers in anatomical study. Paradoxically, health risk among students, instructors, and all involving parties via skin contact and inhalation becomes the major disadvantages of Fd. The immediate symptoms include irritation of the throat, nose, eyes, and skin [3, 8, 14, 15, 18, 19, 21, 22]. The United States Environmental Protection Agency [6] and International Agency for Research on Cancer [11] classified Fd as a human carcinogen, such as nasopharyngeal cancer, ocular melanoma, lung cancer, brain cancer, and leukemia [2, 5, 7, 26]. Accordingly, the Occupational Safety and Health and Administration [17] sets a permissible exposure limit (PEL) of Fd to be 2 ppm for short-term exposure limit (STEL), and 0.75 ppm for time-weighted average (TWA). Since Fd is readily and reactively polymerizing gas at room temperature [1], neutralization of Fd in cadavers to lower its vapor to approximately 1 ppm was the goal of this experiment. Thus far, rigorous and regulatory enforcement of lowering Fd exposure limits have driven anatomists and anatomical institutions to improve laboratory ventilation and exhausting systems, limit the period of laboratory teaching sessions, augment the use of personal protective equipment, and develop advanced preservation method of anatomical specimens, e.g. corrosive cast and plastination. The use of computer imaging, simulation and multimedia is also accepted as an alternative to using cadavers in anatomical teachings. However, their inadequacies in pedagogic principles seems to hinder the substitution for the real cadavers.

Recently, emphasis has been placed on the use of Fd-inactivating chemicals to neutralize or convert Fd in the embalmed cadavers to be the least noxious compound. Some chemical additives, such as cooking salt [4], monoethanolamine, methanol, phenoxyethanol [25], and nitrite pickling salt [12], were claimed to have preservative potential for this purpose. In addition, several expensive commercial fixatives, for examples, Perfect Solution® (Carolina Biological Supply, Burlington, NC, U.S.A.), ESCO Chemicals® (Embalmer's Supply Co., Ontario, Canada), and Streck Tissue Fixative® (Streck Laboratories, Omaha, NE, U.S.A.), are available, but major concerns about using these products at our anatomical teaching laboratory are duration of storing

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the embalmed specimens and embalming cost per cadaver, especially in large animals. Other studies confirmed that ammonium carbonate and ammonium bicarbonate could successfully decrease Fd in cadavers [13, 24]. Although both chemicals yield ammonium ions to react with Fd to form a safe and nonvolatile products—i.e, hexamethylenetetramine or methenamine, they are expensive and not readily available as consumer products. Direct use of ammonia gas, which is a harmful gas and difficult to store or handle, is also unlikely. Instead, nontoxic ammonium ion donors which are commercially available at low cost and are simple to handle, such as urea and urea fertilizer, were evaluated for this particular purpose. In addition, rapid neutralization of Fd using urea-treated dry materials was patented [23]. Our preliminary trials of spraying urea solution (US) or urea fertilizer solution (UFS) directly onto the formalinized specimens or briefly dipping the specimens in the US or UFS could immensely reduce the amount of Fd vapor from the specimens. Covering the specimens with towel soaked with US or UFS could also trap and neutralize the Fd vapor; thus reducing the amount of Fd from vaporizing into the environment. Although urea has been used to reduce risk of exposure to formaldehyde [22], we know of no report of using urea and urea fertilizer in preparing animal cadavers. The present study explored the potential of US and UFS to decrease Fd in chicken cadavers through measuring Fd vapor.

In vitro neutralization: Initially, 32.5% US (QëRC Chemical Co., Ltd., Chonburi, Thailand) and UFS (N-P-K formula 46-0-0, Jiatai Co., Ltd., Bangkok, Thailand) were prepared as stock solutions for diluting to 3, 6, 9, 12, 15, 18, 21, 24, 27, 30 and 32.5%. Five m*l* of each concentration was added into each test tube containing 5 m*l* of 10% FS (QëRC Chemical Co.), respectively. Level of Fd vapor emitting from these mixtures was determined at the mouth of each test tube at 0, 15, 30 and 60 min after mixing by using HAL-HFX105 handled Fd meter (Hal Technology, LLC; Rancho Cucamonga, CA, U.S.A.) for three times. The meter is capable of measuring Fd concentrations ranging from 0–100 ppm with resolution of 0.01 ppm. It was apparently that Fd level was lowered with the increasing concentrations of US and UFS (Fig. 1). Although the neutralizing pattern of these two solutions was similar, UFS could not reduce or neutralize Fd as much as US could. Upon the measurement at 60 min, the lowest concentration of US that could reduce Fd vapor to below 1 ppm (0.74 ppm) was 18% (Fig. 1a), and 27% UFS was needed to decrease Fd to nearly 1 ppm (1.14 ppm) (Fig. 1b). Regardless, 18% US and 27% UFS were selected for the *in vivo* experiment. In addition, pungent odor of Fd was generally undetectable in the reactions having level of Fd vapor lower than 1 ppm.

In vivo neutralization: This experiment received approval from the Animal Ethics Committee of Khon Kaen University, Khon Kaen, Thailand (IACUC KKU No. 3/2015), and was conducted in accordance with the guidelines of the National Institute of Animal Health (Thailand). Accordingly, 30 commercial broiler chickens at 6-week-old weighing 5–7 kg were anesthetized in a diethyl ether chamber, exsanguinated, and then infused 20.8 m*l* of 10% FS per 100 g BW via the right common carotid artery. After being kept for 24 hr at room temperature, the chickens were infused with normal saline (control group, n=10), 18% US (US group, n=10) and 27% UFS (UFS group, n=10) in an equal volume to that of FS. Fd vapor was measured after 48 hr between the thoracic and abdominal cavities. Then, tissues and organs, including superficial pectoralis muscle, iliotibialis muscle, heart, lung, liver, stomach, small intestine, large intestine, and spleen were dissected to investigate the preservation efficacy. Each sample was kept separately in each plastic bag which was tightly closed until the measurement was carried out. The measurement of Fd vapor was performed at the mouth of the bag. Pungent odor of the Fd was noticed at the time of measurement. Initially, normal distribution of the Fd level was examined using Kolmogorov-Smirnov test. The data were evaluated by analyses of variance (ANOVA), and followed by Tukey's HSD for the multiple comparisons of means. Statistical significance for all statistical tests was considered at $P<0.05$. It was apparently that level of Fd vapor in body cavity and each organ after the infusion of US and UFS was decreased considerably (Table 1). Ranges of Fd level were 4.15 ± 0.49 to 7.67 ± 0.63 (mean $\pm$ SD=6.29 ± 1.29) ppm in the control group, 0.21 ± 0.10 to 1.01 ± 0.18 (mean $\pm$ SD=0.73 ± 0.27) ppm in the US group, and 0.49 ± 0.19 to 1.82 ± 0.23 (mean $\pm$ SD=0.98 ± 0.41) ppm in the UFS group. Significant differences were clearly seen between the control and the treatment groups (both US and UFS) at $P<0.05$. Levels of the Fd vapor of the US and UFS treated groups were 8.6 and 6.4 times less than that of the control group, respectively. This statement indicated that neutralizing power of US was about 1.3 times higher than that of UFS. Although US was likely to be more potential than UFS, the pair comparison between the US and UFS group could not found significant difference, except that of the lung, liver, and large intestine (Table 1). Odor of Fd was much less or even unnoticeable in the cadavers or organs with Fd level lower than 1 ppm.

Preservation efficacy: Fd vapor was efficiently eliminated since no pungent odor was noticeable in the neutralized samples, especially those having Fd lower than 1 ppm. The macroscopic observation could reveal that tissues and organs of all groups were well-preserved and suitable for the gross anatomical study. Difference in histological appearances of all tissues among the three groups was not observable. All parenchymal structures of each tissue and organ either in cadavers of the control or the US and UFS groups were easily identifiable (Fig. 2). Minor tissue separations occurred as histological artifacts during the tissue preparation and processing. Thus, this practice should be acceptable as an effective Fd neutralizing protocol in addition to the conventional cadaver preparation.

The remarkable lowering of Fd vapor levels in the *in vitro* experiment could strongly support our confidence on the neutralizing potential of the 18% US and 27% UFS against Fd in the embalm specimens (Fig. 1). Urea and urea fertilizer are nontoxic chemicals yielding ammonia to react with Fd of which harmless and non-volatile methylolurea copolymer (also called monomethylolurea) was formed [10, 16]. Such reaction is usually carried out by using urea and Fd at a molar ratio of 1:1. Odor of Fd vapor after adding US or UFS should be reduced or disappeared; especially in the case that Fd vapor level is lower than 1 ppm [9]. This statement conforms to the odor threshold of Fd of which is 0.5–1 ppm in air. Our results could show clearly that neutralizing power of US is approximately 1.3 times higher than UFS. This would be from the differences in the existing amount of active urea molecules in each solution. Commercial urea fertilizer is usually produced to have only 50% nitrogen to provide nitrogen nutrient to plant and soil [20]. Thus, number of free urea molecules in UFS is approximately one half of that in US with

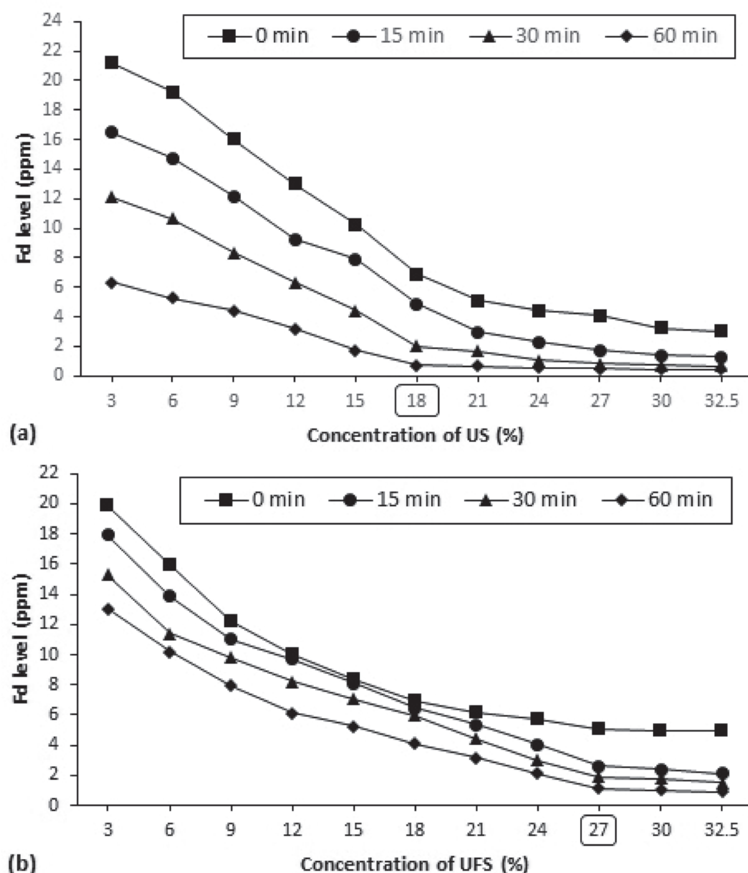

Fig. 1. Level of Fd vapor releasing from the mixtures of 10% FS and various concentrations of US (a) and UFS (b). The measurements were performed at 0, 15, 30 and 60 min after mixing. Apparently, FD vapor was decreased to approximately 1 ppm or lower when 18% US and 27% UFS or higher concentrations of both solution were applied.

Table 1. Level of Fd vapor released from the chicken cadavers with 18% US and 27% UFS treatment. Overall efficacy of 18% US was approximately 1.3 times higher than that of 27% UFS

Samples	Level of Fd vapor (Mean ± SD, ppm)		
	Control (n=10)	18% US (n=10)	27% UFS (n=10)
Body cavity	5.22 ± 0.50[a)]	0.50 ± 0.18[b)]	0.95 ± 0.18[b)]
Heart	7.06 ± 0.52[a)]	0.71 ± 0.17[b)]	0.97 ± 0.13[b)]
Lung	6.91 ± 0.66[a)]	0.68 ± 0.17[b)]	1.39 ± 0.34[c)]
Liver	7.38 ± 0.69[a)]	0.79 ± 0.12[b)]	1.82 ± 0.23[c)]
Stomach	7.67 ± 0.63[a)]	0.82 ± 0.10[b)]	0.94 ± 0.21[b)]
Spleen	4.15 ± 0.49[a)]	0.21 ± 0.10[b)]	0.49 ± 0.19[b)]
Small intestine	6.19 ± 0.66[a)]	0.92 ± 0.18[b)]	0.92 ± 0.16[b)]
Large intestine	6.13 ± 0.52[a)]	1.00 ± 0.14[b)]	0.92 ± 0.10[c)]
Pectoralis muscle	7.38 ± 0.73[a)]	1.01 ± 0.18[b)]	0.88 ± 0.14[b)]
Iliotibialis muscle	4.66 ± 0.49[a)]	0.69 ± 0.10[b)]	0.59 ± 0.13[b)]
Mean ± SD	6.29 ± 1.29[a)]	0.73 ± 0.27[b)]	0.98 ± 0.41[b)]

Differently superscripted letters in each row indicated significant differences at $P<0.05$.

the same concentration. Since 18% US and 27% UFS were used, the difference between number of free urea molecules of both solutions was about 1.29 times. Such difference was quite similar to the 1.3 times difference in neutralizing power of the two solutions. Even all concentrations of US and UFS could react with Fd for 60 min, none could completely neutralize Fd. Many factors that interfered the complete reaction might include temperature, ventilation, equipment, and measuring duration. Further

investigation on these particular aspects may be important.

In this study, a volume of FS being infused into each chicken was calculated from its body weight. This experiment assumed that FS was distributed into each organ or tissue. Nevertheless, the amount of FS distributing into each organ should be dependently upon their size and fluid holding nature—the larger organ or higher fluid holding nature, the higher infusing volume of FS be retained. The differences of Fd vapor levels measured from body cavity and each organ in the control group could be proportional to the volume of free Fd being held inside such organ. A similar reaction and result as in the *in vitro* experiment was expected when 18% US and 27% UFS at an equal volume to FS was infused into the each formalinized cadaver. This statement could be proved by the immense reduction of Fd levels in body cavity and all organs. Initially, the mean value of Fd in the control group was 6.29 ± 1.29 ppm. But after being infused with both neutralizing solutions, Fd levels in the body cavity and most organs of both treatment groups were below 1 ppm, except that in large intestine (1.00 ± 0.14 ppm) and pectoralis muscle (1.01 ± 0.18 ppm) of the US treated group, and lung (1.39 ± 0.34 ppm) and liver (1.82 ± 0.23 ppm) in of the UFS treated group (Table 1). Toxic odor of Fd was much lesser or even undetectable in the treatment groups with Fd level below 1 ppm, especially when compared with the same organs in the control group. Thus, we were confident that both solutions could satisfactorily reduce Fd level to conform to the permissible exposure limits [7]. Upon our embalming experiences, the higher amount of FS levels in these four organs would correlate to their size and fluid-holding capacity. Large intestine is a hollow organ. Its lumen could accumulate FS fluid leaking from the blood vessels during the infusion. To neutralize this excess FS, an extra-amount of neutralizing solution might be required. In poultry, pectoralis muscle is the largest muscle of the body and the whole muscle comprises approximately 8% of the body weight. Hence, FS fluid should be distributed into this muscle in vast amount. As a result, neutralization of Fd in pectoralis muscle would require high amount of neutralizing solution. Lung has a high capacity to hold and accumulate the excess FS fluid in its alveolar lumens; thus, could be considered as the main target of FS deposition. In the cadaver, the infused FS would be able to pass through the alveolar wall to retain in the alveolar lumen. Such retaining FS could release Fd vapor afterward. On this particular, a higher amount of neutralizing solution would be infused to neutralize such excess FS. But with this conventional infusing practice, the amount of solution being infused into lung cannot be easily manipulated. Liver, as a matter of fact, is the largest organ of the body. It might be able to trap high amount of FS inside. Even liver is a solid organ, it has several large blood vessels to drain blood from abdominal viscera, abdomen, pelvic, and hind limb into liver. FS being infused through blood vessel could come to the liver in a high amount. Thus, neutralizing FS in liver to be lower than 1 ppm might need a higher amount of US and UFS than the calculated amount.

In conclusion, 18% US and 27% UFS could be considered as good neutralizers of Fd in cadavers. They

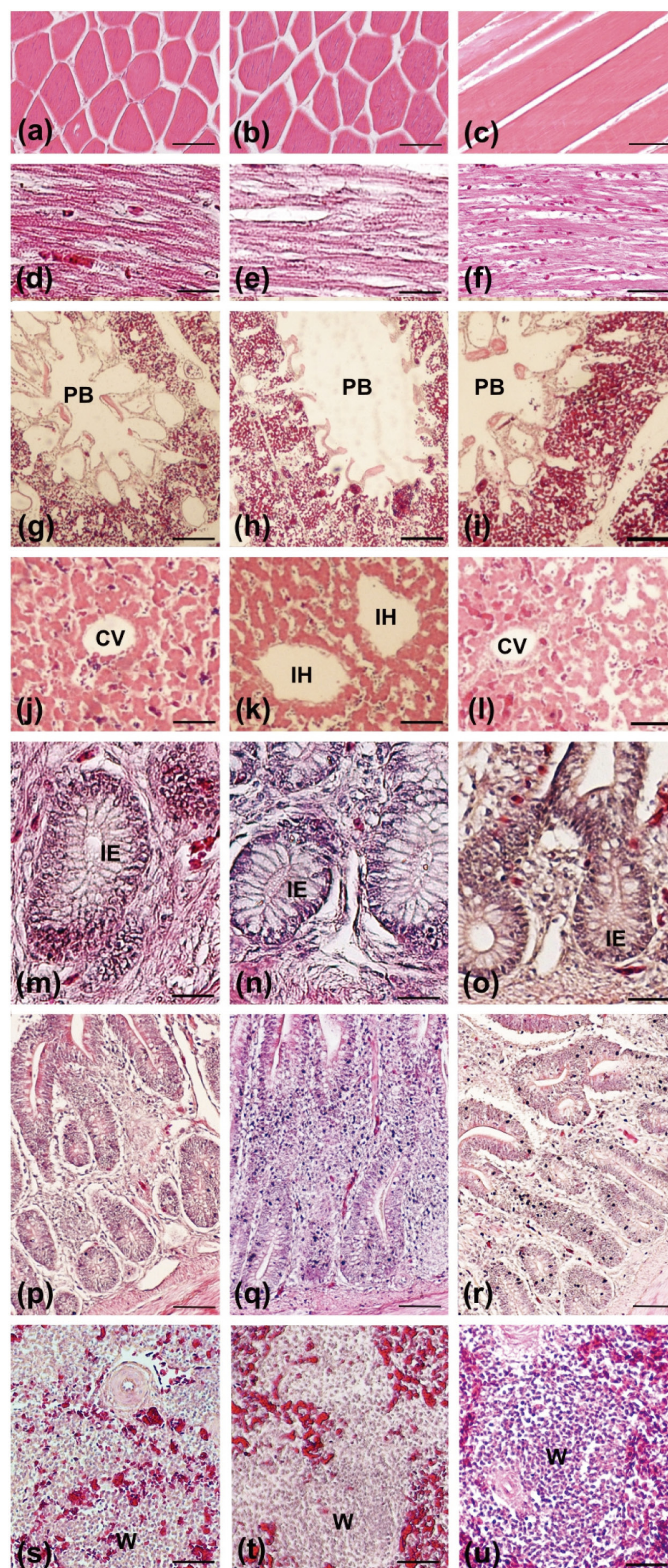

Fig. 2. Histology of pectoralis muscle (a–c), heart muscle (d–f), lung (g–i), liver (j–i), large intestine (m–o), small intestine (p–r) and spleen (s–u) of the three chicken groups—i.e., control (a, d, g, j, m, p and s), US (b, e, h, k, n, q and t), and UFS (c, f, i, l, o, r and u). It was apparently that major parenchymal components of all tissues and organs were well-preserved. CV: central vein, IE: intestinal epithelium, IH: intrahepatic vessels, PB: parabronchus, R: red pulp, W: white pulp. [Bars=50 μm (a–l and p), 20 μm (o, q and s–u), and 10 μm (m and r)].

could reduce pungent and toxic odor of Fd vapor to meet the safety level designated by many organizations without affecting the cadaveric and histological quality. Application of this practice into any formalin-based embalming protocols should be immensely beneficial to all involving parties. Since this is the first report on using US and UFS for neutralizing Fd both *in vitro* and in chicken cadavers, similar study in other animal species is necessary.

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