Biochemical effects of vinyl chloride monomer on the liver of occupationally exposed workers
A.A. Saad,¹ S.M. El-Sewedy,¹ G.A. Bader,² S.M. Mousa ³ and M.M. Mahdy ⁴

ABSTRACT We investigated the effects of vinyl chloride monomer exposure on the liver of 86 workers by measuring β-glucuronidase, arylsulfatase A, adenosine deaminase, 5'-nucleotidase and routine liver function enzymes in the sera of the workers. In 21 of them, three or more of these parameters were raised, with a significant decrease in the level of blood glutathione and a significant increase in the enzyme activity level of glutathione S-transferase. Of these 21 workers, 14 had fatty liver infiltration, 8 of whom were also suffering from liver enlargement. Also, 4 workers had liver enlargement without fatty infiltration and 3 had enlarged spleen. The study highlights the need for vigilance in environmental monitoring and medical surveillance of workers exposed to this chemical.

Effets biochimiques du chiorure de vinylique monomere sur le foie des ouvriers exposes au niveau professionnel
RESUME Nous avons examine les effets du chiorure de vinylique monomere sur le foie de 86 ouvriers en mesurant la β-glucuronidase, l'arylsulfatase A, l'adénosine-déaminase, la 5'-nucéotidase et les enzymes usuels pour la fonction hépatique dans les sérums des ouvriers. Chez 21 d'entre eux, trois ou plus de ces paramètres étaient en augmentation, avec une diminution significative du niveau de glutathion sanguin et une augmentation significative du niveau d'activité enzymatique du glutathion S-transférase. Parmi les 21 ouvriers 14 avaient une infiltration graisseuse du foie, dont 8 souffraient également d'une hépatomégalie. En outre, 4 ouvriers avaient une hépatomégalie sans infiltration graisseuse et 3 avaient une splénomégalie. L'étude souligne la nécessité d'une vigilance avec une surveillance de l'environnement et une surveillance médicale des ouvriers exposés à cette substance chimique.

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Introduction

Epidemiological studies have identified many cases of vinyl chloride-induced angiosarcoma of the liver in people employed in the manufacture of vinyl chloride [7]. Increases in other tumours, including liver, brain, lung, thyroid, lymphatic tissue and skin have also been noted. However, a relationship has not been established between these tumours and vinyl chloride exposure [2].

There seems little doubt that vinyl chloride is mutagenic and carcinogenic as a result of its metabolism by microsomal mixed function oxidases (cytochrome P-450) to chloro-oxirane (chloroethylene oxide) [3]. This highly electrophilic epoxide is a potent mutagen when tested directly or when generated from vinyl chloride in the presence of an appropriate metabolizing system [4]. This observation lead to the present investigation of the biochemical effects of occupational exposure to vinyl chloride monomer (VCM) on the livers of employees of the Egyptian Petrochemicals Company.

Participants and methods

The study was carried out on 106 males ranging in age from 23 years to 41 years, which included a control group of 20 healthy participants with no occupational exposure. The 86 volunteer workers with exposure to VCM were categorized according to where they worked at one of the following four operational sections of the Egyptian Petrochemicals Company.

- Group 1: 25 workers from the chlorine production unit;
- Group 2: 18 workers from the VCM unit (thermal cracking section);
- Group 3: 26 workers from the VCM unit (VCM purification section);
- Group 4: 17 workers from the VCM polymerization unit.

All participants were free from liver diseases. Full clinical and laboratory investigations were carried out to exclude patients suffering from any liver involvement or schistosomiasis, fascioliasis and viral hepatitis. Environmental air sampling was carried out at the different sections of the VCM production process using automatic thermal desorption sample tubes [5]. Blood samples were collected from all participants for assay of the enzyme activities of adenosine deaminase (ADA) [6], arylsulfatase A (ASA) [7], β-glucuronidase (β-glu.) [8], 5’ nucleotidase (5’-NT) [9], and liver function enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) [10], and alkaline phosphatase [9].

The data in all groups were compared by analysing the variance between the parameters studied [11]. The correlation coefficient between the concentration of VCM and enzyme activity levels was also studied [12]. Workers with a high elevation in three or more of the aforementioned biochemical parameters (suspicious group) were investigated by ultrasound examination [13], determination of alpha-fetoprotein (AFP) in serum [14], reduced glutathione (GSH) content in whole blood [15] and glutathione S transferase (GST) activity in serum [16]. The results of this group were analysed using the Student 𝑡-test.

Results

Workers’ average exposure was approximately 1.96 parts per million (ppm) for a maximum of 7.5 years duration. The threshold limit value-time weighted average (TLV-TWA) for VCM is 1 ppm (according to the United States Department of Labor’s Occupational Safety and Health Administration concentration limits for gases).

Results of routine liver function tests were within normal range in all groups of
workers except in Group 4 where alkaline phosphatase activity exceeded the upper normal limit. There was a positive correlation coefficient between the concentration of VCM exposure and the enzymatic activity levels of ADA, β-glu. and 5′-NT in the sera of workers (Table 1). Statistical analyses of the variance between groups (Table 2) revealed a significant increase in the enzymatic activity levels of ADA, ASA, β-glu. and 5′-NT in the sera of the four studied groups compared to the control group. There was a significant increase in the enzymatic activity level of ASA in Groups 2, 3 and 4 compared to Group 1. All workers in the suspicious group had AFP levels within the normal range (0–20 IU/mL) [14].

The t-test analyses (Table 3) revealed a significant increase in ADA, ASA, β-glu., 5′-NT and GST enzymes activity levels in the sera of workers in the suspicious group compared to controls. There was a significant decrease in the GSH content in whole blood of workers in the suspicious group compared to controls. Among the workers in the suspicious group, clinical and ultrasonographic examinations indicated that 14 workers had fatty liver infiltration, 8 of whom were also suffering from liver enlargement. Also, 4 workers had liver enlargement without fatty infiltration and 3 had enlarged spleen.

### Discussion

The present study endeavoured to elucidate the mechanisms by which VCM exposure leads to hepatopathy induction. The results revealed a significant increase in the enzymatic activity levels of ADA, ASA, β-glu. and 5′-NT in the sera of the exposed groups compared to the control group.

It has been reported that the serum level of β-glu. reflects the degree of histological hepatic cell necrosis [17]. There was significant positive correlation between the β-glu. level and the degree of hepatic cell necrosis determined by histological observation. On the other hand, there was no statistical correlation between the transaminase activities and the degree of hepatic cell necrosis [17]. It has been confirmed by immunohistochemical study that the increased β-glu. in serum is released from necrotic hepatic cells into the blood stream [17]. It was speculated that the elevation of serum transaminase activities resulted from the alteration in the membrane permeability of hepatic cells rather than from hepatocellular necrosis [17]. These results suggest that the serum measurement of β-glu. could be used as an indicator to predict the histological progression of hepatitis [17].

It has also been observed that the activities of certain arylsulfatases vary tremendously in some pathological conditions [18]. Thus, elevated activities of these enzymes may indicate an inflammatory process, as it is known from other sources that organs with high metabolic activity [19] and proliferating cells [20] have characteristically high arylsulfatases activity [20].

In past years, there have been numerous reports from many countries demonstrating a significant excess of chromosomal aberrations among workers exposed to vinyl chloride [21]. The evidence for random distribution of spontane-

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>r-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosine deaminase</td>
<td>0.59*</td>
</tr>
<tr>
<td>Arylsulfatase A</td>
<td>0.25</td>
</tr>
<tr>
<td>β-glucuronidase</td>
<td>0.78*</td>
</tr>
<tr>
<td>5′ nucleotidase</td>
<td>0.806*</td>
</tr>
</tbody>
</table>

*Statistically significant.
<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Normal control</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>F-value (P)</th>
<th>Least significant difference</th>
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<tbody>
<tr>
<td>Adenosine deaminase (U/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Mean</td>
<td>503.90</td>
<td>778.90</td>
<td>735.24</td>
<td>736.95</td>
<td>725.27</td>
<td>6.25</td>
<td>Control and other groups</td>
</tr>
<tr>
<td>s</td>
<td>130.83</td>
<td>230.53</td>
<td>364.82</td>
<td>276.25</td>
<td>173.70</td>
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<tr>
<td>s²</td>
<td>29.26</td>
<td>51.55</td>
<td>88.48</td>
<td>60.28</td>
<td>44.85</td>
<td></td>
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<tr>
<td>Arylsulfatase A (U/L)</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Mean</td>
<td>21.85</td>
<td>33.10</td>
<td>27.53</td>
<td>20.29</td>
<td>32.59</td>
<td>10.25</td>
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</tr>
<tr>
<td>s</td>
<td>5.65</td>
<td>11.39</td>
<td>9.38</td>
<td>10.52</td>
<td>12.71</td>
<td>(&lt; 0.05)</td>
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</tr>
<tr>
<td>s²</td>
<td>1.26</td>
<td>2.43</td>
<td>2.27</td>
<td>2.15</td>
<td>3.08</td>
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<tr>
<td>β-glucuronidase (U/L)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>9.10</td>
<td>18.54</td>
<td>20.13</td>
<td>21.13</td>
<td>20.18</td>
<td>4.25</td>
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</tr>
<tr>
<td>s</td>
<td>2.73</td>
<td>8.33</td>
<td>10.17</td>
<td>7.20</td>
<td>5.96</td>
<td>(&lt; 0.05)</td>
<td></td>
</tr>
<tr>
<td>s²</td>
<td>0.61</td>
<td>1.78</td>
<td>2.63</td>
<td>1.47</td>
<td>1.45</td>
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<tr>
<td>5' nucleotidase (IU/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>6.50</td>
<td>12.43</td>
<td>14.71</td>
<td>10.89</td>
<td>12.86</td>
<td>6.25</td>
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<tr>
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<td>8.93</td>
<td>9.35</td>
<td>6.25</td>
<td>6.50</td>
<td>(&lt; 0.05)</td>
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<tr>
<td>s²</td>
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<td>1.86</td>
<td>2.50</td>
<td>1.47</td>
<td>1.74</td>
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</table>

s = standard deviation.

s² = standard error of the mean.
Table 3: Statistical analyses of the biochemical investigations for normal control subjects (NCs) and workers group of suspicious cases (SCs)

<table>
<thead>
<tr>
<th>Measurement</th>
<th>ADA (U/L)</th>
<th>ASA (U/L)</th>
<th>β-gluc (U/L)</th>
<th>5'-NT (IU/L)</th>
<th>GST (IU/L)</th>
<th>GSH (mg%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NCs (n=20)</td>
<td>SCs (n=21)</td>
<td>NCs (n=20)</td>
<td>SCs (n=21)</td>
<td>NCs (n=20)</td>
<td>SCs (n=21)</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>504.0</td>
<td>920.0</td>
<td>22.0</td>
<td>36.0</td>
<td>9.0</td>
<td>24.0</td>
</tr>
<tr>
<td>s</td>
<td>131.0</td>
<td>323.0</td>
<td>6.0</td>
<td>15.0</td>
<td>3.0</td>
<td>9.0</td>
</tr>
<tr>
<td>s_ε</td>
<td>29.0</td>
<td>76.0</td>
<td>1.0</td>
<td>4.0</td>
<td>0.6</td>
<td>1.9</td>
</tr>
<tr>
<td>r^2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.11</td>
<td>3.88</td>
<td>7.53</td>
<td>5.29</td>
<td>3.84</td>
<td>-6.48</td>
</tr>
<tr>
<td>p^b</td>
<td></td>
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<tr>
<td></td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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</tbody>
</table>

* - test for comparison between workers group of suspicious cases and controls.
* - Probability value for comparison between workers group of suspicious cases and controls.
ADA = adenosine deaminase.
β-gluc = β-glucuronidase.
ASA = arylsulphatase.
GST = glutathione S-transferase.
5'-NT = 5' nucleotidase.
s = standard deviation.
GSH = reduced glutathione.
s_ε = standard error of the mean.

Our study demonstrates that occupational exposure to vinyl chloride may damage the liver. Hepatic injury developed insidiously and no disturbances were observed in routine liver function examination. About 76% of the parameters studied were normal in the workers exposed to vinyl chloride in the present study. This observation may explain the significant increase in enzyme activity levels of both ADA and 5'-NT in the sera of these enzymes. The increased levels of these enzymes might reflect the lineages of specific lymphocyte populations. The activity of 5'-NT enzyme has been found to be higher in B cells than in T cells. ADA is an enzyme with cell maturity [26], and to correlate with the stage of cell maturation. Studies have indicated that the levels of these enzymes correlate with conventional immunologic markers [24].
ehrome P-450) to chloro-oxirane (chloro-
roethylene oxide) [3]. This epoxide has been
found to be a potent mutagen when tested
directly or when generated from vinyl chlor-
ide in the presence of an appropriate metab-
olizing system [4]. The other known
mutagenic metabolite of VCM is chloroa-
cetaldehyde, the rearrangement product of
chloro-oxirane (chloroethylene oxide) [29].
The major detoxification pathway for these
two mutagenic metabolites is by conjugation
with GSH [30]. The enzymes catalysing
these reactions are called glutathione S-
transferases and are present in high amounts
in liver cytosole and in lower amounts in
other tissues [31]. If the potentially toxic
xenobiotics were not conjugated to GSH,
they would be free to combine covalently
with DNA, RNA, or cell protein and could
thus lead to serious cell damage [32]. It has
been reported that the blood level of GST
[33] or ligandin [34] increases in human liver
disease because it is assumed to escape
from liver cells into the blood under patho-
logical conditions [16]. Marked increase in
serum GST is observed when there is se-
vere degeneration or necrosis of liver cells at
the time of blood collection [16]. The afore-
mentioned observations are in line with our
findings which indicate the increased en-
zeyme activity level of GST in the blood of
the suspicious group.

Clinical and ultrasonographic examina-
tions in our study indicated that 14 workers
had fatty liver infiltration, 8 of whom were
also suffering from liver enlargement; 4
workers had liver enlargement without fatty
infiltration and 3 had enlarged spleen. In
a previous study, histopathological obser-
vation of the liver of workers exposed to
VCM in low doses revealed reactive hepatis-
tis, slight inflammatory cell infiltration [35],
Kupffer cell activity and focal necrosis,
with or without fat accumulation in the liv-
er [35]. This offers a good explanation for
the present biochemical results.

These observations highlight the need
for continual vigilance with environmental
monitoring and medical surveillance of
VCM-exposed workers. The depletion of
GSH content in whole blood levels associ-
ated with highly significant increases in the
enzymatic activity levels of β-glu. and ASA
persisting in the long term may severely af-
flect workers’ health. High levels of β-glu.
and ASA indicate a predisposition to cancer
that could be due to the liberation of the
active carcinogens from glucuronide and
sulfate conjugates. The diagnostic criteria
of chemical hepatic injury are therefore
proposed to alert medical professionals, in-
dustrial hygienists, safety personnel and
factory inspectors in order to avoid or de-
lay early occupational liver diseases.

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