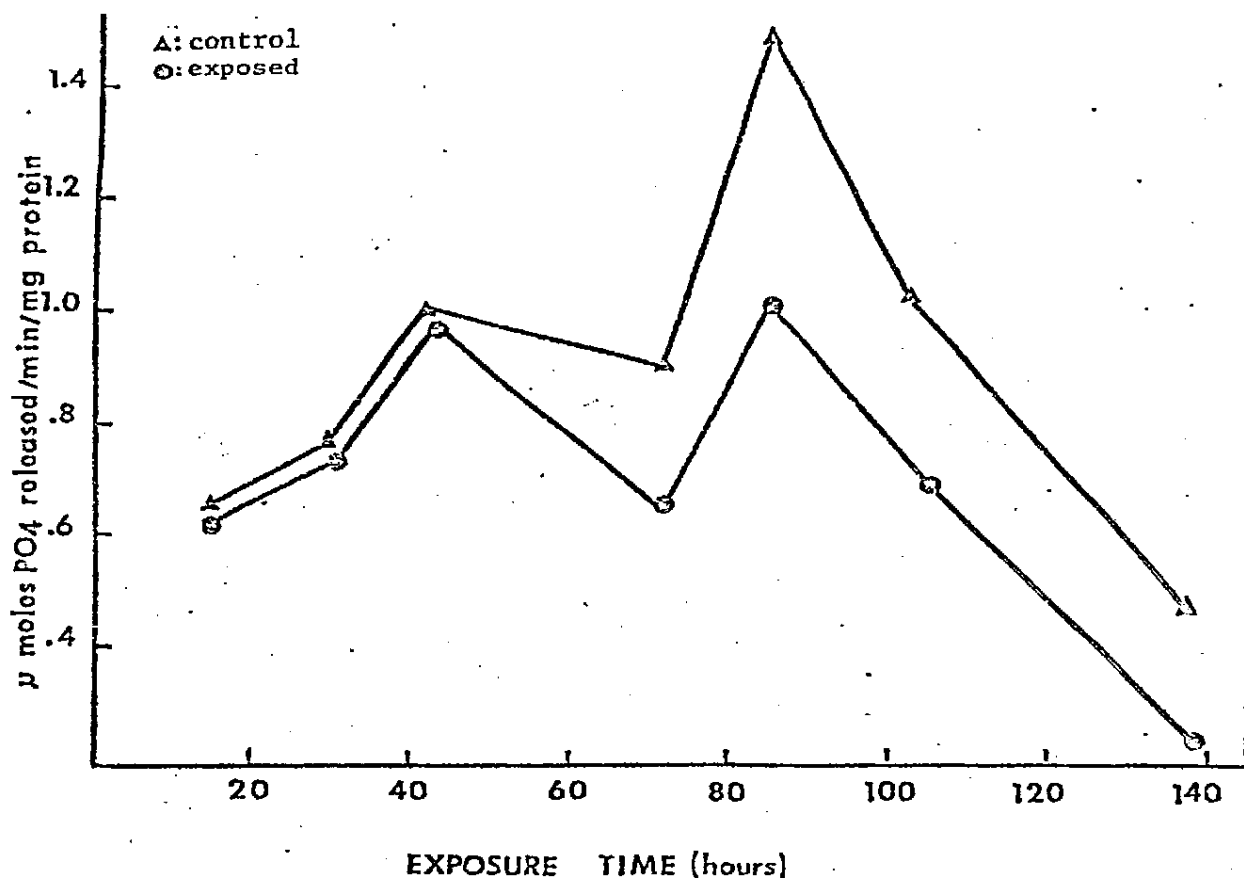


SLIDE 2, 3, 4, 5.

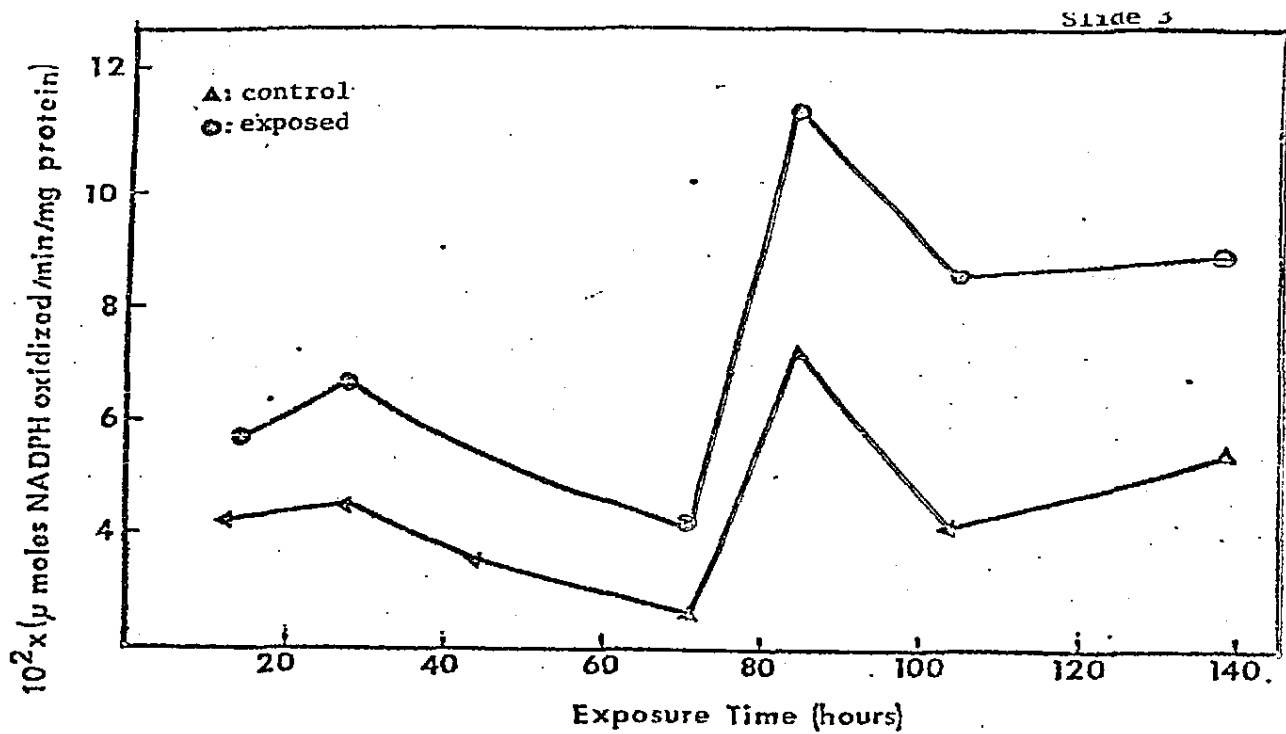
This illustrates an early increase in glutathione reductase activity followed closely by a fall in glucose-6-phosphatase which becomes significant after 71 hours of total exposure (Slide 3). At 100 hours, a marked increase in glucose-6-phosphate dehydrogenase (Slide 4) similar to that seen in metabolic studies by Weber and his group concerning hepatic primary cell neoplasms was found. As shown in Slide 5, Weber's molecular correlation concept of neoplasia shows an impairment of the glucose-6-phosphatase producing a decrease in gluconeogenesis and an increase in the pentose phosphate shunt pathways which eventually lead to an increase in purine biosynthesis and nucleic acid synthesis. This, presumably, leads to increased liver cell replication. These biochemical changes in the early stages of vinyl chloride exposure may well explain the histological findings in humans exposed to vinyl chloride which appear to be characteristic of vinyl chloride exposure. These histological findings of focal nodular hyperplasia of the hepatocytes may well be the histological reflection of a shift in gluconeogenesis and an increase in nucleic acid synthesis, induced by a mechanism not yet completely understood during the early phases of vinyl chloride exposure. If one were to carry this to a logical conclusion one would presume that the hepatocyte would eventually develop into a malignant cell with continuous regeneration and exposure to carcinogens. The fact that the primary hepatocyte does not become malignant in the adult rat, but does become malignant in the newborn rat with similar vinyl chloride exposure, led us to study the detoxifying capabilities of the hepatocyte versus the sinusoidal lining cells. During this time, there were no significant differences in the usual biochemical clinical studies used for screening early vinyl chloride injury.

VVC 000003275



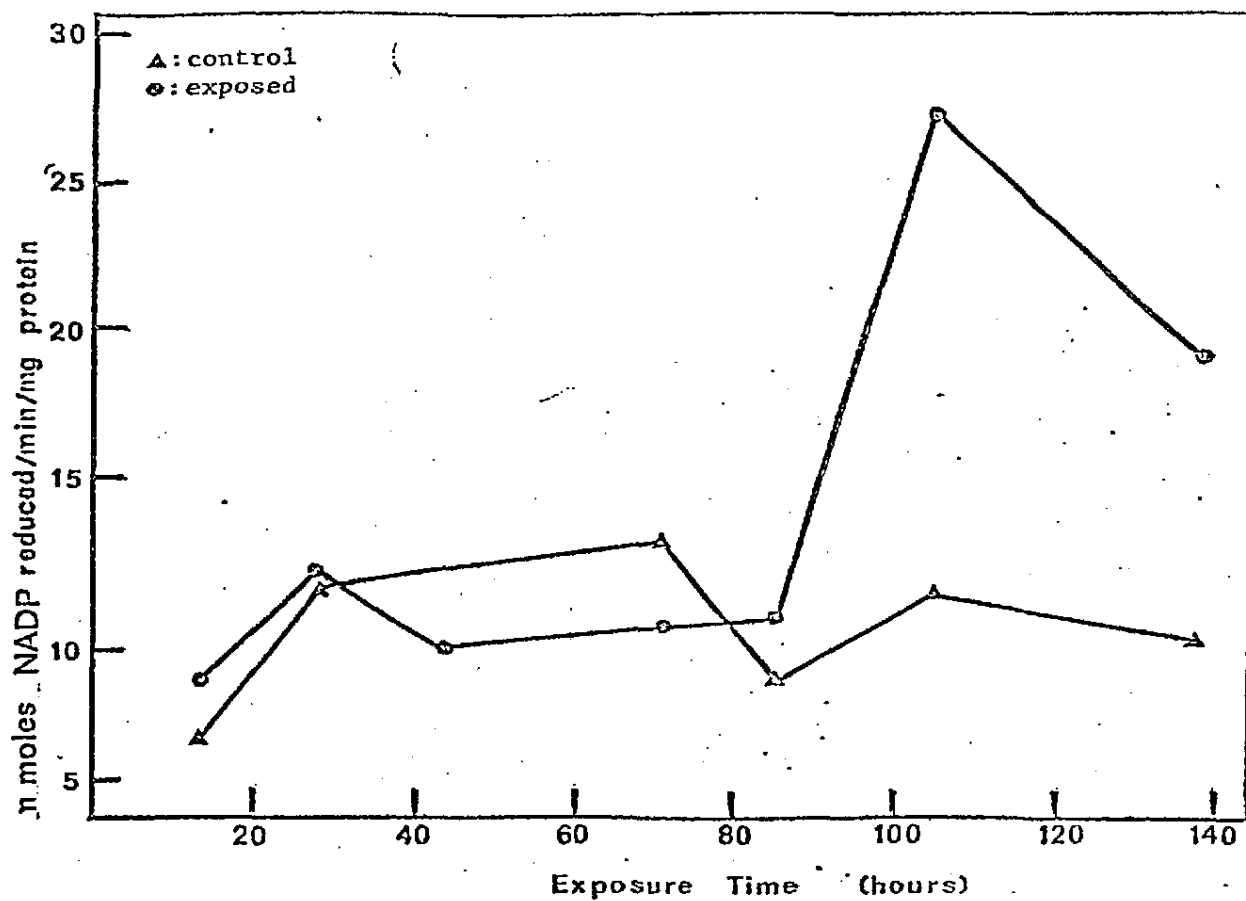
SLIDE 2 SHOWS THE GROUP MEANS OF THE SPECIFIC ACTIVITY OF GLUCOSE-6-PHOSPHATASE WITH RESPECT TO TIME OF EXPOSURE. THE CONFIDENCE INTERVALS FOR GROUP MEAN DIFFERENCES ON THE ACTIVITIES OF ENZYMES DISCUSSED ARE BASED ON THE ERROR MEAN SQUARE FROM A TWO FACTOR ANALYSIS OF VARIANCE WITH INTERACTION. THE TWO MAIN EFFECTS BEING TIME IN HOURS AND EXPOSURE-NONEXPOSURE. THERE IS NO SIGNIFICANT DIFFERENCE BETWEEN THE EXPOSED AND CONTROL GROUPS FOR THE FIRST THREE TIME POINTS. HOWEVER, THE MEAN LEVEL OF THE EXPOSED GROUP IS SIGNIFICANTLY LESS THAN THAT OF THE CONTROL AFTER 71 HOURS OF EXPOSURE.

VVC 000003276



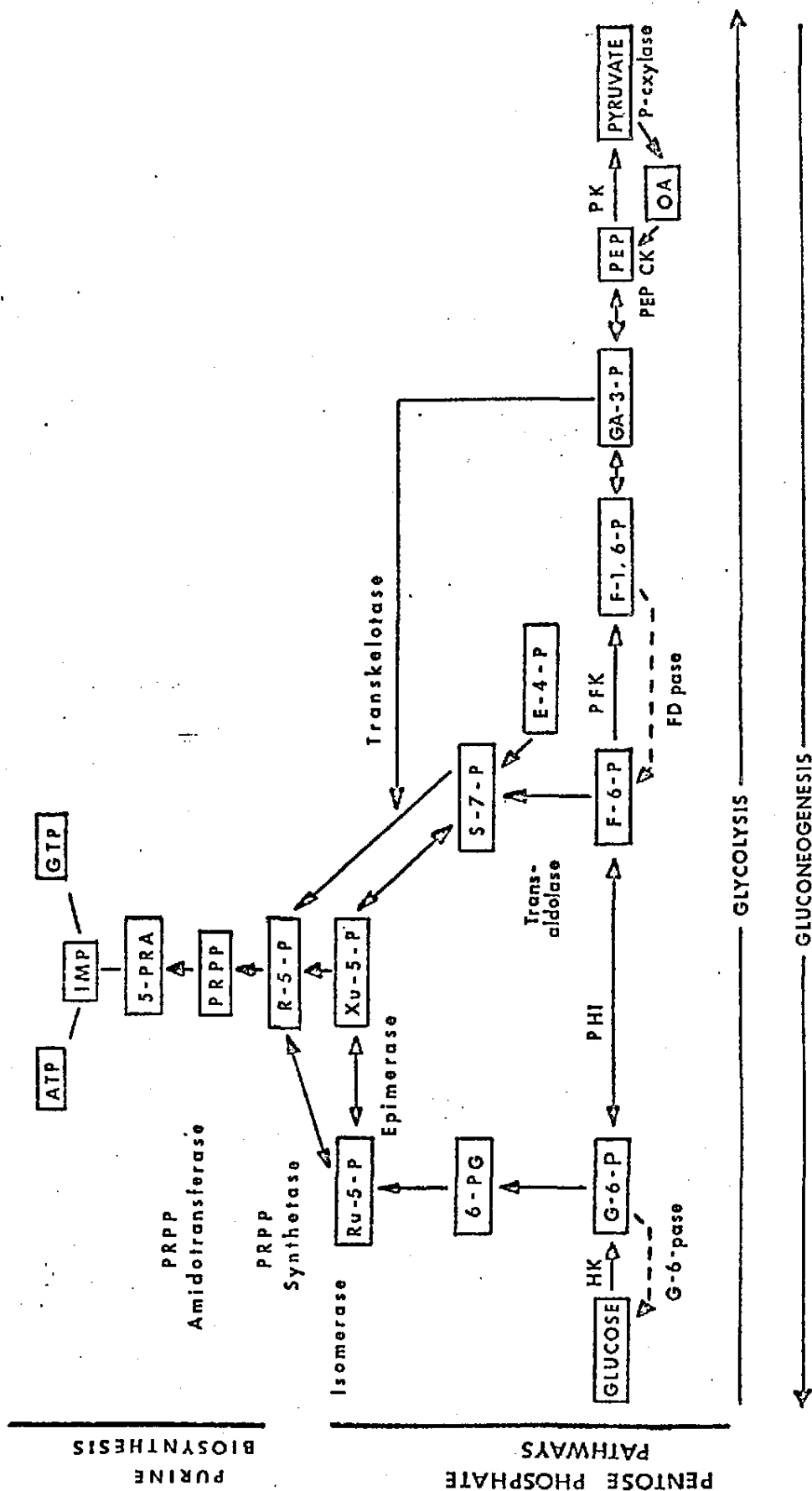
SLIDE 3 SHOWS THE GROUP MEANS OF THE SPECIFIC ACTIVITY OF GLUTATHIONE REDUCTASE WITH RESPECT TO TIME OF EXPOSURE. THERE IS A SIGNIFICANT INCREASE THROUGHOUT THE ENTIRE EXPERIMENT.

VVC 000003277



SLIDE 4 SHOWS THE GROUP MEANS OF THE SPECIFIC ACTIVITY OF GULCOSE-6-PHOSPHATE DEHYDROGENASE WITH RESPECT TO TIME OF EXPOSURE. THERE IS A SIGNIFICANT INCREASE AFTER ABOUT 100 HOURS OF EXPOSURE.

VVC 000003278



VINYL CHLORIDE OXIDATION AND DETOXIFICATION

J.T. Du, Ph.D., and C.H. Tamburro, M.D.

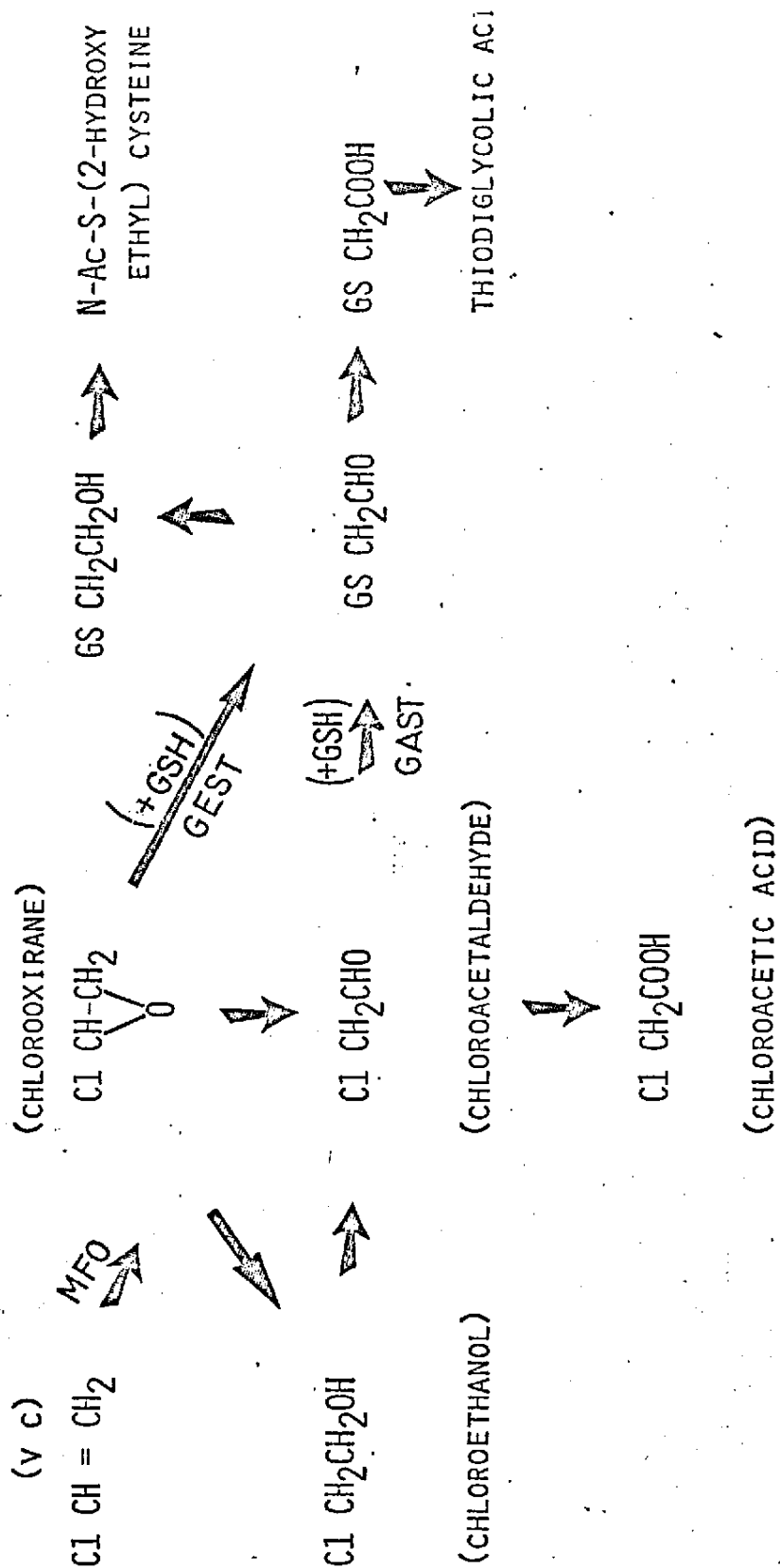
The second phase of study of the biochemical changes associated with vinyl chloride exposure were directed at oxidation and detoxification. Sprague-Dawley rats were randomly exposed to controls and vinyl chloride exposure at 28,000 ppm, 7 hours per day, five days per week, for a total of 210 hours of exposure, for six weeks duration. Slide 1 shows the vinyl chloride metabolic fate scheme presently under use. Vinyl chloride being metabolized at higher levels by mixed-function oxidase within the liver may be converted to chlorooxirane or the epoxide. This unstable intermediate may then spontaneously revert to form chloroethanol and the chloroethanol may further be metabolized to chloroacetaldehyde. Secondly, the chlorooxirane may be directly detoxified by GSA via the glutathione epoxide S-transferase or further oxidized to chloroacetaldehyde. Chloroacetaldehyde in turn may be shown by glutathione aralykly-S-transferase or further oxidized to chloroacetic acid. Studies by Wong and Streips have already shown that vinyl chloride, chloroethanol and chloroacetic acid have no mutagenic properties in bacteria. However, the chlorooxirane and the chloroacetaldehyde are both mutagenic. Animal studies demonstrated significant differences in the liver of vinyl chloride exposed animals who showed no physical or clinical abnormalities. These findings are listed in Slide 2. They include an elevation of non-protein sulfhydryl content, (Slide 3) glutathione reductase, (Slide 4) glutathione epoxide-S-transferase (GEST) (Slide 5) and glutathione aralykly-S-transferase (GAST) (Slide 6) concomitant with the increase in glucose-6-phosphate dehydrogenase and reduction in P450 content (Slide 7).

A composite of the studies from 14 to 350 hours is shown in Slide 8 which illustrates the earliest increases occurring in glutathione reductase followed later by an increase in glutathione content. It is interesting to note that the increase in GEST occurred before the increase in GAST around 70 to 80 hours of the total exposure. This implies that the chlorooxirane accumulation is to be

handled by direct detoxification by the glutathione but with prolonged exposure more is converted to the chloroacetaldehyde which then is further detoxified by the GAST. These data are compatible with other findings of increased amounts of chloroacetic acid in the urine of individuals with very high prolonged exposures. This would imply that at initial doses the vinyl chloride is able to be metabolically handled and properly detoxified and that there may be a number of mechanisms by which the active metabolites are handled within the hepatocyte thus, preventing it from inducing DNA injury and subsequently angiosarcoma. Whether these metabolic capabilities are also present in the sinusoidal lining cells has lead us to our more recent studies.

VVC 000003281

DETOXIFICATION WITH GLUTATHIONE

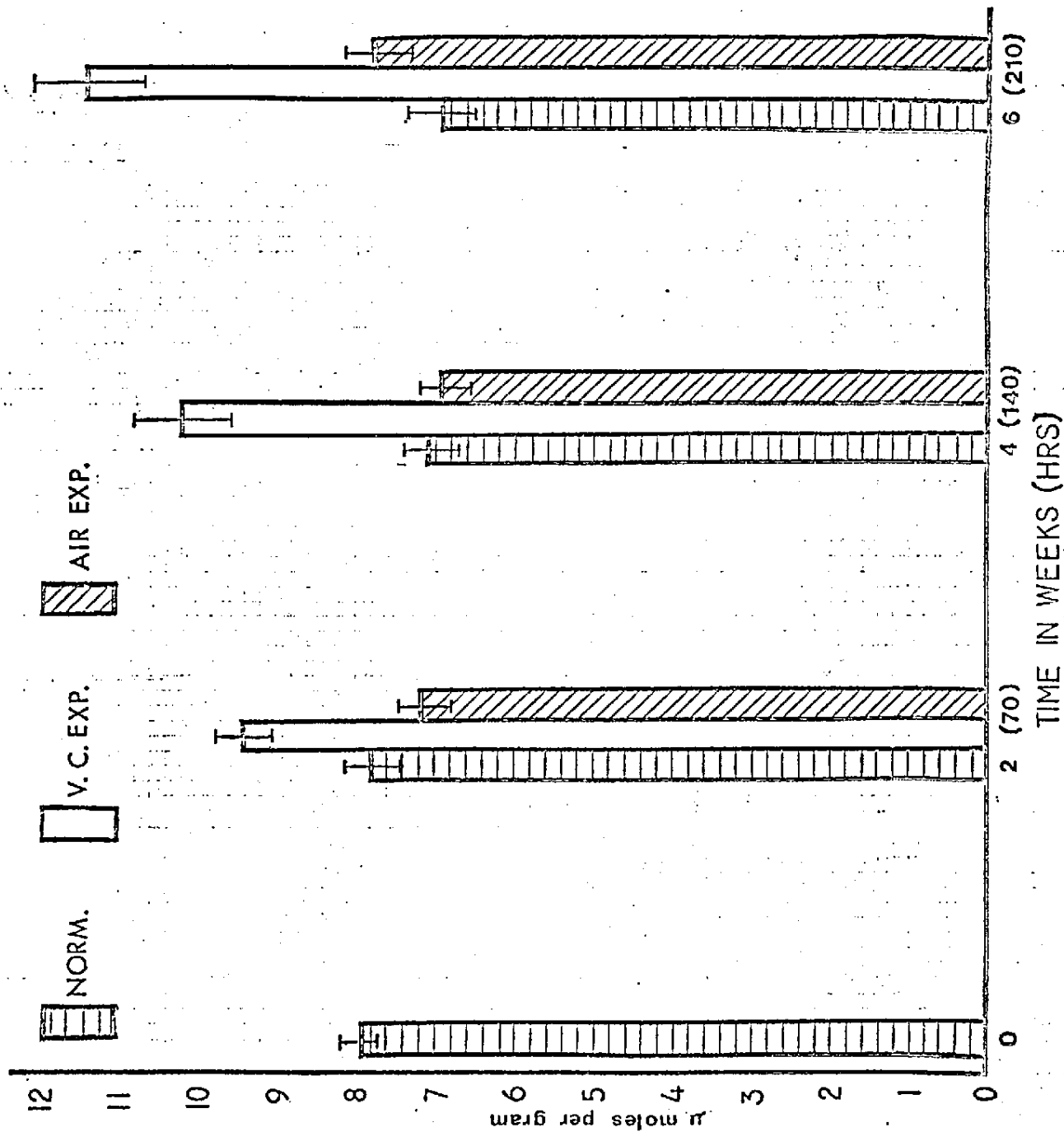


VINYL CHLORIDE METABOLIC FATE

RESULTS

IN COMPARISON WITH THE TWO CONTROL GROUPS, THE FOLLOWING STATISTICALLY SIGNIFICANT DIFFERENCES ($P < 0.05$) WERE FOUND IN THE LIVER OF THE VINYL CHLORIDE EXPOSED BEFORE ANY SIGN OF PHYSICAL OR CLINICAL ABNORMALITIES WERE SEEN.

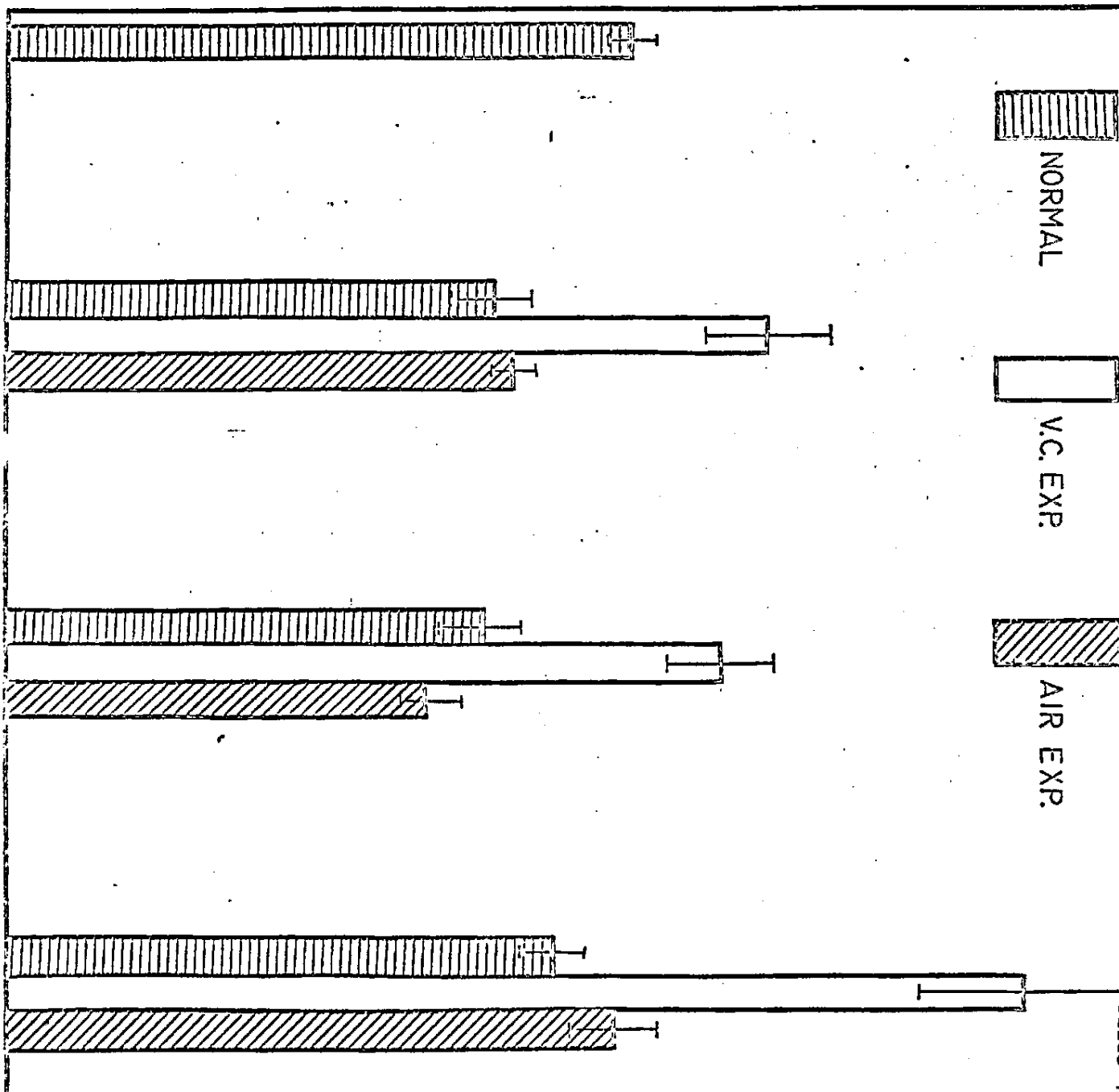
1. AN ELEVATION OF NON-PROTEIN SULFHYDRYL CONTENT.
2. AN ELEVATION OF GLUTATHIONE REDUCTASE.
3. AN ELEVATION OF GLUTATHIONE EPOXIDE -S- TRANSFERASE (GEST).
4. AN ELEVATION OF GLUTATHIONE ARALKYL -S- TRANSFERASE (GAST).
5. A REDUCTION OF P-450 CONTENT.
6. AN ELEVATION OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE



LIVER GLUTATHIONE REDUCTASE

.100 x μ moles per minute per mg protein

0 1 2 3 4 5 6 7 8 9 10



NORMAL

V.C. EXP.

AIR EXP.

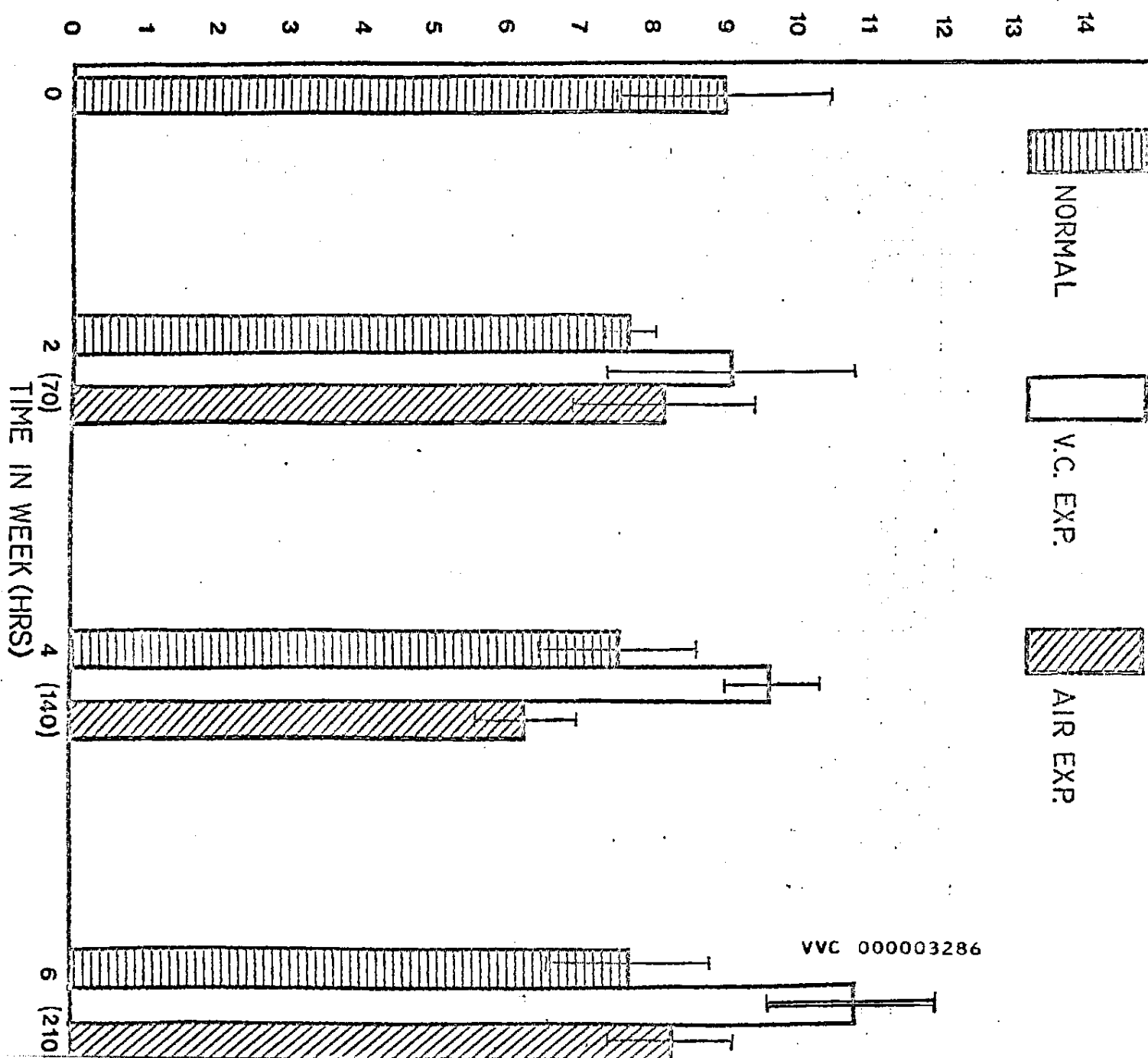
Slide 4

VVC 000003285

LIVER

GLUTATHIONE EPOXIDE -S- TRANSFERASE

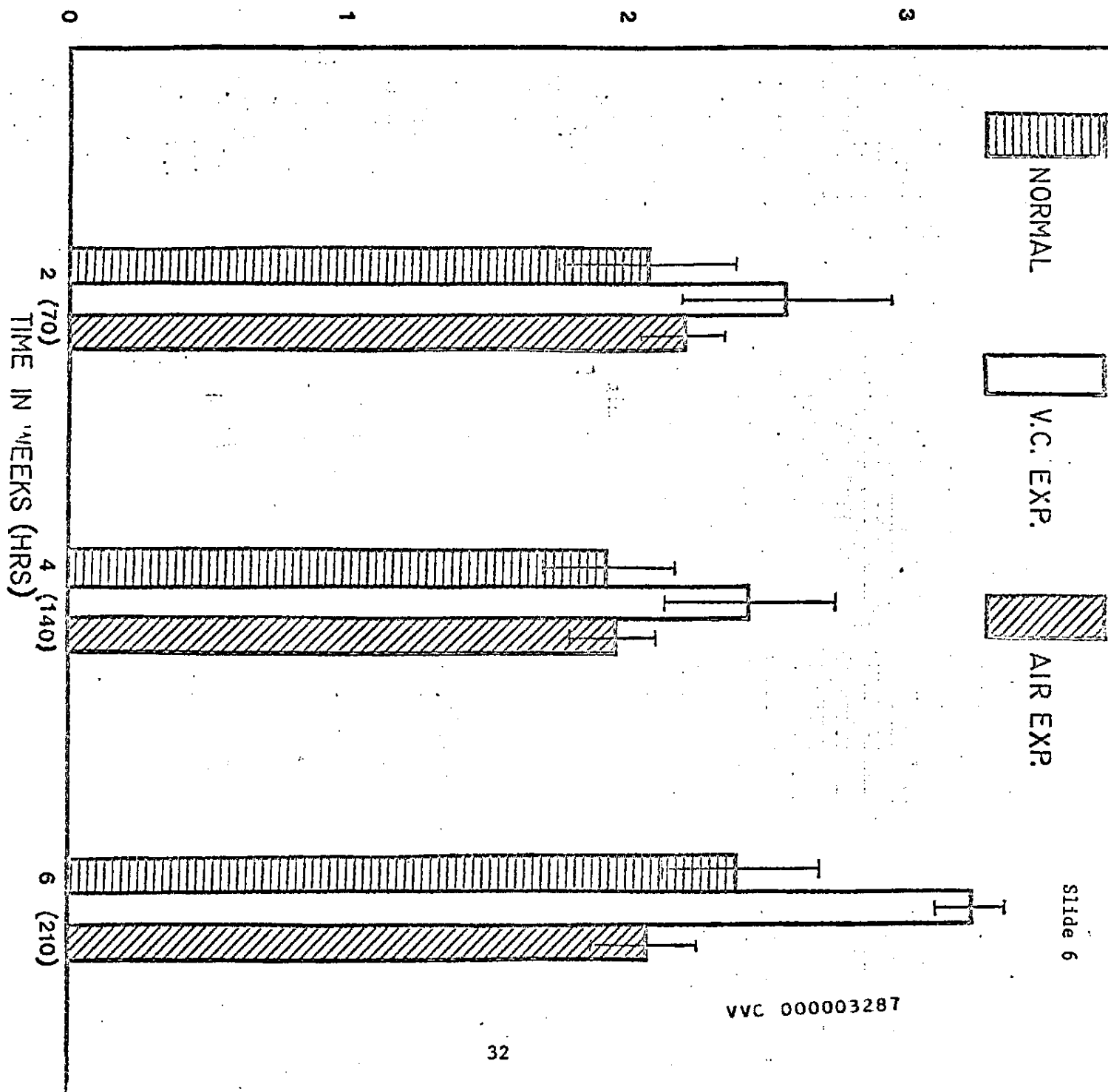
100 x μ moles per minute per mg protein



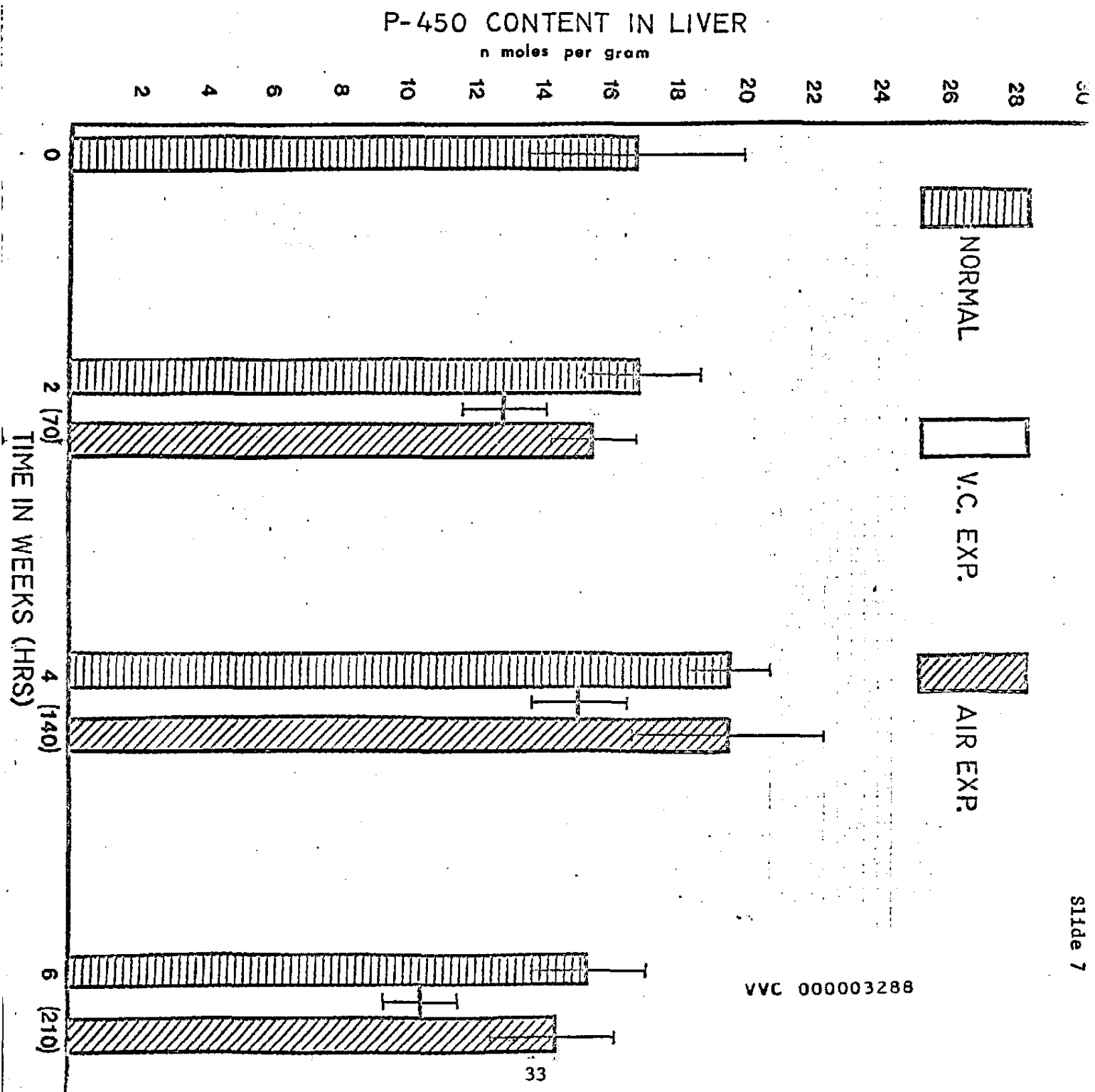
LIVER

GLUTATHIONE ARALKYL - S-TRANSFERASE

10 x μ moles per minute per mg protein



VVC 000003287



% Increase above Control

