## ACKNOWLEDGEMENTS

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## ABSTRACT

After partial hepatectomy, the vitamin C content of the liver of the rat, studied during 11 days at room temperature showed a cyclic phenomenon which corresponded to the restorative activity as reported by Higgins et al. The same cyclic phenomenon was observed for non-adapted partially hepatectomized rats exposed to cold (~5°C) over a period of 7 days and for the adapted partially hepatectomized rats exposed to cold over a period of 9 days.

The resistance of partially hepatectomized rats to cold (~5°C) was found to vary according to the time elapsed between the operation and the moment of exposure to cold. The ones that were exposed 48 hours after operation showed a remarkable survival (80%) as compared to the sham-operated (50%) and to the non-operated controls (10%) and also as compared to the partially hepatectomized ones exposed to cold at any other chosen time (3, 6, 12, 24 and 72 hours) after operation, at end of 14 days in the cold.

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INTRODUCTION

It is known that the liver of the white rat undergoes restoration after partial hepatectomy, resulting from partial or complete blocking of blood vessels leading to a definite lobe or lobes of liver, or from chloroform and $CCl_4$ necrosis of the liver or from partial surgical removal of the liver under normal conditions (1-10). It is also established that the restoration of the liver is the result of hypertropy and hyperplasia of the remaining part of the liver in the case of clumping of the blood vessels or partial surgical hepatectomy (1-10). Besides, the effect of partial hepatectomy on the resistance of rats to cold has been shown by Desmarais et al (11, 12) to be detrimental in the case of animals not adapted or preexposed to cold and to have comparatively no effect on the animals preexposed to cold 48 hours before operation. On the other hand Weiss et al (13) have recently shown that cold (5°C.) has no effect on the restoration of the liver and that partial hepatectomy has no effect on the resistance of animals (rats) to cold. In the literature no mention has been given to the effect of partial hepatectomy on the level

of ascorbic acid of tissues which has been shown by Dugal et al (14) to be important in resistance to cold and beneficial for acclimation. The object of the present investigation was to find out if these various effects of partial hepatectomy on animals could be related to its effect on the ascorbic acid content of tissues especially of the liver. The following approaches were adopted to test our objective or hypothesis:

1. To determine how the ascorbic acid content of the restoring liver behaved during a period of 11 days at room temperature.

2. To see if cold (-5°C.) has any detectable effect on the restoration and on the ascorbic acid content of the restoring liver in adapted and non-adapted animals.

3. To study the resistance of animals to cold (-5°C.) during the different phases of liver restoration.

LITERATURE REVIEW:

I. Regeneration of liver

The ability of the liver to regenerate or restore was first observed by Cruveilhier and Andral (1) and later by Calcucci, Carona, Tozzoni and Griffini (1) though the era of experimental pathology was first opened for investigators according to modern standards by Von Padwyssozki (2). The latter after removal of wedges of liver from rat, cat, guinea pigs and rabbit noted evidence of restoration in adjacent hepatic cells. The first scientific description of regenerating liver was given by Ponfick (3). By removing 75% of the liver from rabbits and dogs he observed a threefold increase of the remaining hepatic tissue and described the new hepatic cells as swollen, more voluminous with pale bright cytoplasm and with somewhat larger nuclei than normal. Besides he detected the occurrence of mitosis by the second day. Ponfick's observations were duplicated and confirmed by Von Meister (6) in rats, rabbits, and dogs and by Mann and Magath in dogs (4). Von Meister observed that cells near the center of the lobules

remained in a latent stage due to pressure from the strong growth at the periphery. Hall (5) has confirmed that the regenerative activity started at the periphery and proceeded to the center of the liver.

The literature on liver regeneration up to 1929, revised extensively by Fishback (6) indicates that three main methods were used to study the regenerative activity of the liver. The first method was by ligation of the hepatic artery and vein. Regeneration of the liver was observed after ligation of the hepatic artery or after ligation of the hepatic vein or of both (6). The second method used was the production of necrosis by prolonged chloroform anesthesia. Regeneration of the liver after necrosis by the chloroform was observed to occur within 2 or 3 weeks by Whipple and Sperry and later confirmed by Schultz, Hall and Baker (7). Fishback (6) himself employed the third widely used method, the partial surgical removal of the liver. By removing one-fifth to three-quarters of the liver from dogs, he observed regeneration of the remaining part of the liver by hypertrophy and hyperplasia starting from the peripheral zones.

The first quantitative study of the regeneration of the liver was made by Higgins and Anderson (8). According to them the restoration of the liver of the rat started on the latter part of the first day after operation and a more rapid rate of restoration occurred during the third and fourth days after partial surgical removal of the liver in terms of liver weight per 100 gr. of body weight. They have also observed that the regenerating liver exhibited a cyclic activity. On the other hand, Brues et al (9) viewing the restoration of the liver in terms of cell increment have noted that the increase in cell number only starts 24 hours after operation. The increase in cell number was found by them to follow the same rate as the mass increase as found by Higgins except that no secondary acceleration was observed.

The effect of age on the restoration of the liver after partial hepatectomy was considered by Morris et al (10). They found that the liver of rats at all ages studied showed a remarkable ability to restore after partial hepatectomy. However, the rate and the total amount of liver regeneration was observed to vary inversely as the age of the animal.

Dugal et al (15) studying the effect of partial hepatectomy on the spontaneous activity of the white rat have observed that the initial spontaneous activity was regained 96 hours after operation.

How the liver regenerates has not yet been clearly understood. However, different investigators have endeavoured to unwrap the mechanism involved.

Novikoff et al (16) examining the biochemical changes during liver regeneration found that at the time of most rapid growth the concentration of pentose-nucleic acid (PNA) associated with protein synthesis increased while no significant changes occurred in the concentration of other substances normally related to growth like lactic acid, ATP, ADP, adenylic acid and free pentose phosphate where the concentrations are compared with the initial concentration. Brues and co-workers (9) have observed that the nitrogen concentration of the regenerating liver though low on the first day after operation (1.2%) rapidly approached the nitrogen concentration of the normal liver 4 days after operation thus corroborating Novikoff's findings.

Norris and his colleagues (10) comparing the aerobic and anaerobic glycolysis production of embryonic, neonatal and regenerating rat liver have shown that the

rate of anaerobic glycolysis of regenerating liver was not increased over that of the normal resting liver in rats of comparable age and was independent of the length of time after partial hepatectomy and of the total amount of regeneration. On the other hand, they have reported that the rate of anaerobic glycolysis of embryonic rat liver was conspicuously higher than that of the neonatal liver and that the rate for neonatal liver was in turn increased over that of normal adult liver.

Higgins (17, 18) was inclined to think that the factors involved in the restoration after partial hepatectomy might be vascular as well as functional (physiological). He observed a more rapid increase in liver weight in rats when partial hepatectomy was accompanied by ligation of the common bile duct and by splenectomy. He also observed that in the chicken when the vena cava was ligated above the kidney, thereby increasing the venous blood flow to the hepatic portal vein and consequently to the remnant liver, the restoration of the liver occurred even after removal of the left lobe. Stephenson (19) by combining partial hepatectomy with partial ligation of the portal

vein in white rats, was able to prevent to some extent the restoration that follows simple partial removal of the liver. Also, Bell (20), in 1926, has described regeneration in the livers of dogs after production of atrophy by ligation of the common bile duct followed by removal of the obstruction causing atrophy. According to him, the degree of destruction of parenchymal cells and the extent of proliferation of connective tissue was roughly proportional to the duration of obstruction of the common bile duct.

McJunken and Breuhaus (21) have demonstrated that in rats subjected to partial hepatectomy, injection of homologous macerated liver greatly increased the regeneration of hepatic cells and they concluded that the protoplasm of cells (hepatic) contains a cell formative stimulus.

The amount of reducing substances in tissues during regeneration was considered by Rynkina (22). He reported that the tissues of the extremities of axolotles contain about 6 mg. per cent of ascorbic acid. The tissues of regenerated extremities contain twice as much ascorbic acid even as long as 60 days after regeneration has been completed. The ascorbic acid

concentration remained high after the proteolytic activity had decreased. There was a direct relation between the increase in proteolytic activity and the decrease of oxidized glutathione. The decrease in proteolytic activity during the later stages of regeneration was related to the appearance of the oxidized form of glutathione and indicated protein synthesis. At the same time ascorbic acid increased.

Davidson et al (23) have reported that the total nucleic acid concentration as measured by nucleoprotein phosphorus is high in pregnancy, very high in the embryo, rises on fasting and is not appreciably altered in regenerating liver, while acid soluble nucleotide was found unaltered on fasting and increased in regenerating liver.

## II. Biosynthesis of Vitamin C

Ever since the controlled experiment by Lind (24) in 1753 when he cured scurvy by administration of orange and lemon juices, the agent causing scurvy has been studied extensively. In 1907 Hoest and Frolich (24) produced scurvy experimentally in guinea pigs. In 1932 vitamin C was isolated from

lemons by Waugh and King (24) and was found to be identical with a compound obtained from adrenal gland and from cabbage in 1928 by Szent-Gyorgyi. The structural formula of vitamin C was established in 1933 by Hyworth and Hirst (24). As far as the synthesis of vitamin C is concerned it was found that the vitamin is synthesised by certain moulds, fungi, all higher plants and by animals except guinea pigs, primates, and man (24).

The ability of the rat to synthesize vitamin C from dietary sources has been shown both in vivo and vitro via chemical titration and bioassay. The vitamin is found in all organs of the rat with varying concentrations. The adrenals and corpus luteum have the highest concentration followed by the brain, the liver, the testes, the ovaries and other glandular tissues and muscles. On the other hand Sutton et al (23) endeavouring to determine the site of ascorbic acid synthesis in rats were inclined to think that the synthesis of ascorbic acid is not a specific function of any single gland but more probably a result of general metabolism. From their experiments they have shown that though the concentration of vitamin C is high in the ovary (corpus

luteum), pituitary and adrenal glands, the removal of all these organs did not enhance any significant drop in vitamin C excretion of chloretone-treated rats. In fact, Burns (26) has labelled the microsomes of liver as the probable site of ascorbic acid synthesis.

The vast knowledge about the biosynthesis of ascorbic acid in rats has been the result of using certain drugs like chloretone, chloral hydrate or certain barbituric acid derivatives which have been shown by Longencker et al (27) to increase the biosynthesis of the vitamin and also its urinary excretion. Stimulation of the intermediary metabolites of ascorbic acid by the drugs was offered as a probable role and function of chloretone and other similar drugs. Jackel et al (23) using labelled glucose and administering it into chloretone-treated rats of the Wistar strain obtained approximately 0.3% converstion of glucose $C^{14}$ from glucose to ascorbic acid within 24 hours. They have established that the total transfer of $C^{14}$ from glucose to ascorbic acid within 24 hours was approximately equivalent to the total conversion of dietary carbohydrate to ascorbic acid. Smythe et al (29) carrying a study of the synthesis of ascorbic acid by slices of

liver, kidney and brain from normal rats and from rats that had been stimulated to excrete large amounts of ascorbic acid have shown that the tissues from treated rats (fed chloretone) showed evidence of synthesis in vitro while those from normal rats showed distinctly less or no evidence of synthesis. Liver and kidney tissues exhibited a greater activity than brain or muscle.

On the other hand, Ruffo and co-workers (30) have given evidence of the synthesis of vitamin C in vitro. They obtained negative result when rat tissues were substituted by guinea pig tissues. Also Guha et al (31, 32, 33) have reported the synthesis of Vitamin C in vitro by rat tissues (spleen, kidney, and liver) incubated with mannose for 3 hours at pH 7.4 at 37°C. Besides, they have duplicated their in vitro findings in vivo by injecting mannose intravenously. In almost all their papers, Guha and his colleagues have dogmatically held that mannose is the precursor of vitamin C. This leads us to the consideration of the precursor and the mechanism of vitamin C synthesis.

Though different investigators have disputed

on the identification of the precursor of ascorbic acid as either mannose (Guha et al, 31, 32, 33) galactose and glucose (Burns (35), King, (37), Isherwood et al, (34) yet each investigator has suggested that the carbon skeleton of L-ascorbic acid in both plants and animals is formed from a hexose sugar. The mechanism of biosynthesis of L-ascorbic acid in rats has been shown by Isherwood et al (34) and Burns (35) to follow the pathway herewith:-

D-glucose---→ D-glucuronic acid---→ L-gulonic acid---→ L-gulonolactone---→ L-ascorbic acid.

Burns (35) has even shown that ascorbic acid is an intermediary metabolite in carbohydrate metabolism in animals. The inability of man, monkey and guinea pig to synthesize ascorbic acid was explained by Burns (35) as being due to the lack of a liver enzyme system required for the conversion of L-gulonolactone to L-ascorbic acid. Grollman et al (36) have confirmed Burns' observations that the enzyme (Enzyme III) necessary to convert gulonate to ascorbic acid is present only in the liver of animals which are able to synthesize ascorbic acid. From their experiment they have recognized three enzymes in the biosynthesis of L-ascorbic

acid from D-glucuronic acid, the reactions being:-

a) D-glucuronate + TPNH + $H^+$ $\rightleftharpoons$ (Enzyme I) L-gulonate + $TPN^+$

b) L-gulonate + $DPN^+$ (Enzyme II) $\longrightarrow$ (3 keto-L-gulonate) + $H^+$ + DPNH

c) (3 keto-L-gulonate) (Enzyme III) $\longrightarrow$ L-ascorbate + $H_2O$

They have also reported that organs like the kidneys, heart, spleen, and the brain of animals synthesizing ascorbic acid and most of the organs of animals which do not synthesize ascorbic acid contain large amounts of Enzyme I and Enzyme II. They concluded that the inability of these organs to synthesize ascorbic acid was due to the lack of Enzyme III rather than to an excessively high rate of destruction of L-ascorbic acid.

Horowitz and King (37) and Burns and Evans (38) have pinpointed the precursor of L-ascorbic acid as D-glucuronolactone or L-gulonolactone using labelled D-glucuronolactone (Horowitz et al and Burns) and L-gulonolactone (Burns et al).

Relation of ascorbic acid to resistance and acclimation to cold

The relation of cold acclimatisation and resistance to cold to ascorbic acid in mammals has

been studied by Dugal and his co-workers (14, 39, 40 41). In rats exposed to cold over a period of 4 to 6 months, Dugal and Thérien (14) observed a considerable increase in ascorbic acid content of the tissues especially the adrenals (80%) regardless of the diet. They have also reported that the increase in ascorbic acid content of the tissues was much smaller if the rats exposed to cold received ascorbic acid daily. In the case of the rats which died in the cold, they found that the tissues had a low content of ascorbic acid.

The importance of vitamin C in resistance to cold observed in the rat were confirmed in guinea pigs and monkeys (animals which are not able to synthesize vitamin C). In guinea pigs, Dugal and Thérien (14) have observed that resistance and adaptation to cold depended on the amount of ascorbic acid received daily and that the animals needed more and more ascorbic acid as the temperature of the surroundings was being lowered. Greater retention of ascorbic acid in tissues, especially in adrenals at low temperature and failure to acclimate to cold due to decrease in ascorbic acid content of the tissues has been reported by Dugal and Thérien (14)

via tissue analysis and urinanalysis (39).

As far as the monkeys are concerned, Dugal and Fortier (40) observed that monkeys (rhesus macacus) preexposed to mild cold (10°C.) during six months and receiving daily 25mg. of ascorbic acid do not resist intense cold better than the controls kept at room temperature receiving the same daily dose of ascorbic acid. On the other hand they have reported that monkeys exposed to the same cold environment but receiving a dosage of 325mg. of ascorbic acid daily showed a better performance in resisting cold than controls kept at room temperature and also better than the group preexposed to cold and receiving a daily dosage of only 25mg. of ascorbic acid. The criterion for the resistance to cold was the ability to maintain their rectal and intramuscular temperatures and the ability of the monkeys to avoid frost bite when exposed suddenly to -20°C. The drop in Respiratory Quotient (R.Q.) of monkeys (Rhesus Macacus) exposed to cold was reported by the same authors (41) to be independent of the dosage of ascorbic acid administered daily.

## MATERIALS and METHODS

### General

Over 800 male albino rats of the Wistar Strain were used. At the start of each experiment, rats 8-10 weeks old weighing 150-250 grams were used since from these ages on, the level of vitamin C of the liver was found to be constant for all practical purposes, (42). The rats were individually caged, fed Purina Laboratory Chow and water ad libitum. All of the experiments were completed within a year starting June, 1959 and finishing June, 1960.

### Techniques

a) Partial hepatectomy

Partial hepatectomy was made under ether anesthesia with the customary precautions. A midline incision was begun at the level of the xiphoid appendage and extended 3 cms down. The xiphoid cartilage was removed enabling the free protrusion of the median and lateral lobes of the liver. By applying slight pressure on either side of the incision the

median and the lateral lobes were delivered through the abdominal wall. After severing the hepatic ligaments a heavy thread was tied around the vessels and the ducts of the two lobes and then the latter were disected out. Any blood spilled off during dissection was removed from the peritoneal cavity by gauze moistened with isotonic NaCl solution. The surgical wound was closed with thread sutures. The excess blood from the liver was removed by blotting using filter paper and carefully weighed for determination of vitamin C (L-ascorbic acid).

The controls underwent sham operation which involved the removal of the xiphoid appendage, the delivery of the median and lateral lobes through the incision under ether anesthesia, and the return of these lobes into the abdominal cavity. Autopsy was performed under almost the same conditions of anesthesia to minimize the enhancing effect of ether on the vitamin C content of the liver; this effect has been observed by Bowman (43) and Beyer et al (44). In fact, in our attempt to find suitable anesthesia, we tried nembutal in a preliminary experiment and found

that it had enhancing effect on the level of vitamin C of the liver which interfered with the normal vitamin C content of the liver. Besides, the recovery period with nembutal was considerably longer than with ether.

At autopsy the liver was removed while the animal was still under ether anesthesia. This operation included the damaging of the hepatic blood vessels and of the vena cava and the opening of the diaphragm which eventually led to the death of the animal and the removed liver was weighed. The sham operated controls underwent similar operative protocol as the patially hepatectomized rats except that their livers were weighed in two parts; (i) the part corresponding to the remaining liver of partially hepatectomized animals, and (ii) the total liver. The non-operated controls were sacrificed by breaking their necks, and their livers were weighed the same way as the sham operated controls. In both sham operated and non-operated controls most of the time the part corresponding to the remaining liver after partial hepatectomy was used for determination of vitamin C.

b) <u>Restoration percentage</u>

To determine the percentage of the remaining

liver after partial hepatectomy 25 male albino rats weighing 200 to 350 grams were used. It was found that the remaining liver after surgery constituted 34.0%±2.6. The mass of the liver left at surgery in partially hepatectomized animals was calculated by employing the formula used by Weiss et al (13).

$$P.W.L.L. = W.L.R. \times \frac{0.340}{0.651}$$

where P.W.L.L. is the estimated weight of liver mass left at surgery, and W.L.R. the weight of removed liver. As we have already mentioned the liver mass both at operation and at autopsy was removed under anesthesia and hence we did not include the correction introduced by Weiss et al (13) for the variation of liver mass due to anesthesia.

Therefore, Regeneration $\% = \left(\frac{W.L.L. - P.W.L.L.}{P.W.L.L.}\right) \times 100$

where W.L.L. is the liver mass at autopsy.

c) Determination of Vitamin C (L-ascorbic acid)

Vitamin C (L-ascorbic acid) was determined using the dye method as outlined by Bessey and King (45) with slight modifications. Twenty tables of the dye 2, 6 dichlorophenol-indophenol were crushed and dissolved in 250 ml. of redistilled water. The dye was

standardised with 20 mg. of L-ascorbic acid dissolved in 100 ml. of a mixture of 4% meta-phosphoric acid and 8% trichloroacetic acid used for extraction of L-ascorbic acid from the liver.

The removed liver, both at operation and at autopsy, was put in 50 ml. of a mixture of 4% meta-phosphoric acid and 8% trichloroacetic acid. Three to four hours elapsed in every case before the liver was removed from the mixture to be grinded in a mortar. All of the mixture, that is 50 ml. was added to the grinded liver and the suspension was centrifuged. A 10 ml. aliquot was titrated with the standardised 2, 6 dichlorophenol-indophenol until a faint pink colour lasting at least 15 seconds was observed. A micro-burette of 10 ml. capacity with a precision of 0.05 ml. was used. Freshly prepared dye was used most of the time. Dye left over was kept in the refrigerator not more than 2 days and was always standardised with freshly prepared L-ascorbic acid when used.

With this method, only L-ascorbic acid is determined; consequently all our results on vitamin C determination refer specifically to L-ascorbic acid

which is thought to be the state in which vitamin C is mostly found in the liver of the rat (42).

In every series of experiments, the vitamin C concentration of the removed liver at operation served as the control. The significant variation of vitamin C concentration at any given time after operation was determined using the "t" test.

## First Series of Experiments

### Introduction

As already stated, the purpose of our investigation was to find out if the various and apparently contradictory effects of partial hepatectomy on resistance of animals to cold could be correlated to its effect on the vitamin C content of tissues especially of the liver. To achieve this aim, it was, therefore, necessary to determine first the vitamin C content of the restoring liver at room temperature (20±2°C.). Consequently the purpose of this first series of experiments and our first experiment was to find out how the vitamin C content of restoring liver behaved over a period of 11 days at room temperature.

The duration of our experiment was set at 11 days because according to Higgins (8) the liver is essentially restored between 10 and 14 days after operation. Also from our own experience the level of vitamin C, as we shall see later, was normal at about 10 to 11 days after partial hepatectomy.

## Methods and Materials

Two-hundred-and-fifteen male albino rats of the Wistar strain weighing 200-270 grams were used in this experiment. Ninety-five of them were partially hepatectomized, 40 sham operated and 80 served as non-operated controls. These were divided into eleven groups each group consisting of:

a) 7-10 partially hepatectomized rats,

b) 3-5 sham operated rats, and

c) 6-10 non-operated controls.

The eleven groups were sacrificed at an interval of 24 hours until the 11th day after operation. In each case at operation and at autopsy the removed liver was weighed carefully and used for determination of vitamin C, and at autopsy it was also used for determination of percentage of restoration of the liver.

## Results and Discussion

The vitamin C (L-ascorbic acid) concentration of the restoring liver in mg/gm of wet of the liver at room temperature exhibited a cyclic variation, as could be seen from Table I and Figure 1. Twenty-four hours after partial hepatectomy, the vitamin C concentration dropped abruptly; then after 48 hours it started to rise.

On the 3rd day the vitamin C concentration seemed to approach the initial vitamin C concentration which was found at the moment of surgery to be 0.25±0.01 mg/gm of wet weight of the liver. On the 4th day the vitamin C concentration showed a drop and this level was maintained till the 8th day. The period from the 8th day till the 11th day showed a further increase in vitamin C concentration. The sham operated and non-operated controls showed no significant change in vitamin C content during the whole duration of the experiment. The values of vitamin C content obtained for sham operated and non-operated controls were 0.27±0.01 mg/gm of wet weight and 0.23±0.00 mg/gm of wet weight of liver respectively.

Figure 2 shows percent of restoration. Maximum restoration seemed to occur between the 2nd and 4th day and this is quite in accord with Higgin's finding (8). After the 4th day the restoration seemed to hit a plateau and remained so until the end except for the 10th day for unknown reasons.

The cyclic phenomenon exhibited by the vitamin C concentration of the restoring liver corresponded to the cyclic restorative activity of the liver reported

by Higgins et al (8). According to them the peak of restorative activity was found to occur on the third day and the period from the fourth till the seventh day was described as cytologically dormant except for a large uptake of water. The period from the 8th till the 14th day was marked by secondary acceleration in restorative activity. As we have already described the undulating or cyclic phenomenon of vitamin C concentration of restoring liver follows the same pattern as the restorative activity. It is evident, that there appears a close relation between the restorative activity and the level of vitamin C of the restoring liver. This close relation suggests a possible role of vitamin C in liver restoration, most probably mediated through its strong biological oxidation-reduction reaction coupled with cytochrome system (46). When one considers Szent-Gyorgyi's (46) theory of vitamin C action as a "Hydrogen carrier", the contemplation that vitamin C plays a role in restorative activity of the restoring liver does not seem farfetched.

## TABLE I

### Restoration and Vitamin C content of the Restoring Liver at Room Temperature

| AT OPERATION | | | | AT SACRIFICE | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| No. of rats used | Body wt. grs. | Wt. of liver removed grs. | Calc.wt. of liver remaining grs. | No. of rats | Body wt. grs. | LIVER REMAINING Wet wt. grs. | Restoration | L-ASCORBIC ACID Mg/gm of wet weight | (P) | Hours after operation |
| 10 | 254±7* | 6.15±.34 | 3.30±0.18 | 10 | 230±6 | 4.46±.18 | 35.7±6.2 | 0.18±0.01 | 0.[illegible]1 | 24 |
| 10 | 213±2 | 5.64±.14 | 3.02±0.07 | 10 | 204±2 | 5.25±.12 | 74.9±6.3 | 0.22±0.01 | 0.005 | 48 |
| 9 | 212±3 | 5.07±.21 | 2.72±0.11 | 9 | 204±4 | 6.22±.16 | 131.1±8.5 | 0.23±0.01 | 0.10 | 72 |
| 10 | 215±2 | 5.12±.15 | 2.75±0.08 | 10 | 214±3 | 7.69±.13 | 181.6±7.3 | 0.16±0.01 | 0.001 | 96 |
| 9 | 221±4 | 5.14±.16 | 2.75±0.08 | 9 | 228±3 | 8.43±.21 | 207.0±6.9 | 0.18±0.01 | 0.001 | 120 |
| 9 | 230±6 | 5.40±.19 | 2.89±0.11 | 9 | 242±7 | 9.22±.40 | 220.7±15.7 | 0.19±0.01 | 0.001 | 144 |
| 9 | 224±4 | 4.92±.27 | 2.64± .12 | 9 | 232±6 | 8.44±.38 | 221.7±12.3 | 0.17±0.01 | 0.001 | 168 |
| 8 | 215±4 | 5.20±.27 | 2.79± .14 | 8 | 225±5 | 8.82±.34 | 219.2±12.3 | 0.18±0.01 | 0.001 | 192 |
| 7 | 223±3 | 5.30±.21 | 2.84± .11 | 7 | 249±6 | 8.85±.28 | 215.4±19.4 | 0.23±0.02 | 0.20 | 216 |
| 7 | 238±6 | 5.30±.20 | 2.84± .11 | 7 | 240±8 | 7.61±.29 | 168.3±.8 | 0.28±0.001 | 0.20 | 240 |
| 7 | 253±2 | 5.44±.20 | 2.92± .11 | 7 | 275±4 | 8.93±.32 | 207.9±13.5 | 0.26±0.01 | 0.70 | 264 |

* standard deviation

** L-ascorbic acid at time 0 = 0.25±0.01 mg/gm of wet wt.

P Level of significance.

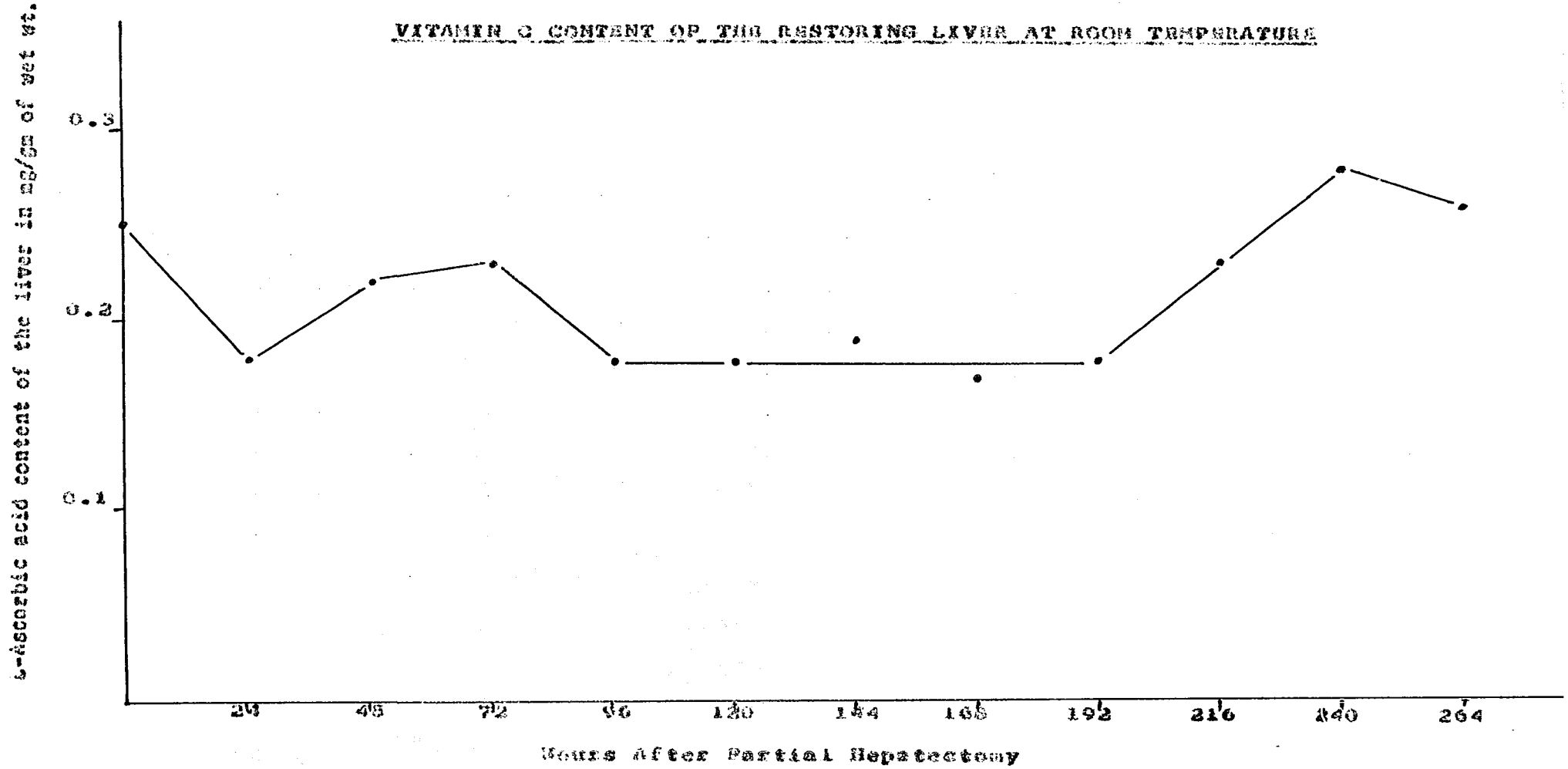

VITAMIN C CONTENT OF THE RESTORING LIVER AT ROOM TEMPERATURE
L-ascorbic acid content of the liver in mg/gm of wet wt.
0.3
0.2
0.1
24
48
72
96
120
144
168
192
216
240
264
Hours after Partial Hepatectomy

Fig. 1

RESTORATION OF THE LIVER OF THE RAT AT ROOM TEMPERATURE

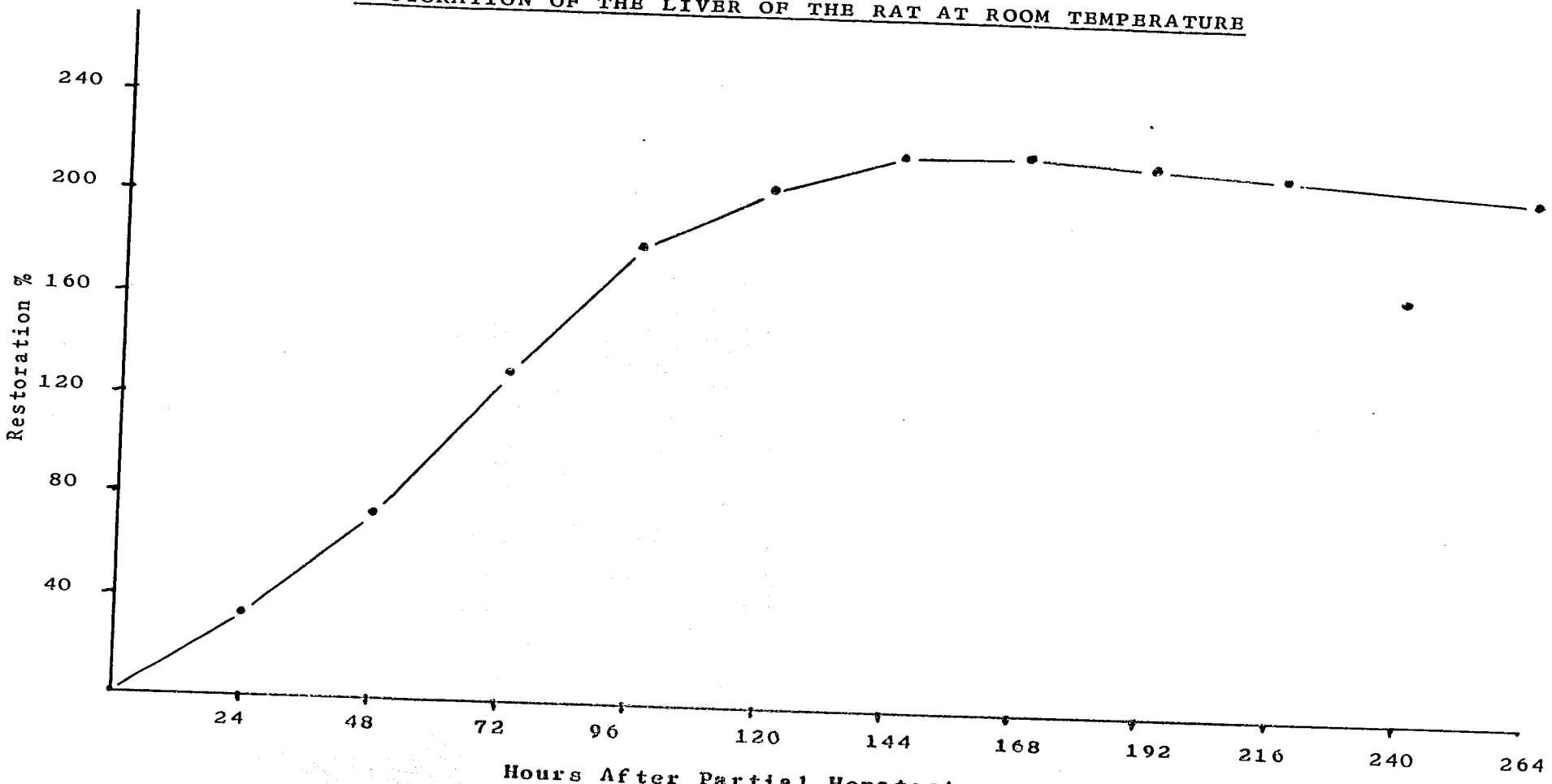



Fig. 2

Second series of experiments.

Introduction

In our first series of experiment we have already noted the close relation that exists between the restorative activity as described by Higgin et al (8) and the vitamin C content of the liver. Knowing this, we wanted to see how the resistance of partially hepatectomized rats to cold was affected during the different phases of restorative activity, especially during the first 3 days. Hence the following experiments were aimed at showing the resistance of partially hepatectomized rats to cold during the periods of 3, 6, 12, 24, 48 and 72 hours following partial hepatectomy.

Materials and Methods

One-hundred-and-eighty male albino rats of the Wistar strain weighing 200-300 grams were employed in this experiment. One-hundred were partially hepatectomized, 60 were sham operated and 20 served as general non-operated controls for all groups. The partially hepatectomized and sham operated rats were divided into 6 groups and each group consisted of:

a) 10-20 partially hepatectomized rats

b) 10 sham operated control

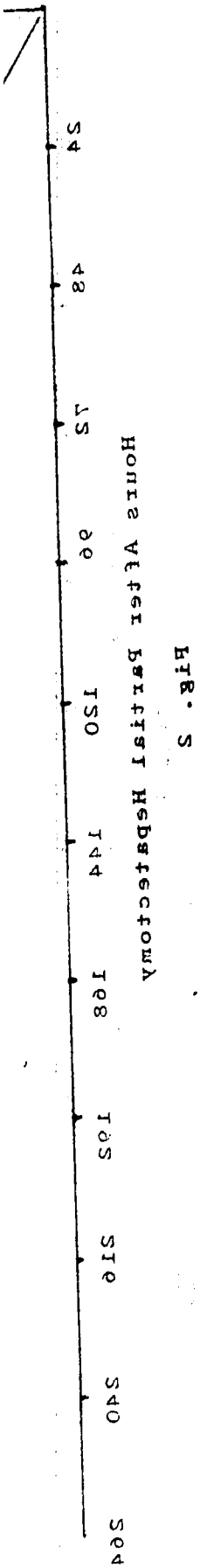



Fig. 5

These 6 groups were exposed to cold (-5°C.) 3, 6, 12, 24, 48 and 72 hours respectively after operation. The experiment lasted 14 days during which time the survival was recorded daily and the change in body weight was registered every day of the first week and every other day of the second week.

Results and Discussion

a) Survival (Table III, Figs. 3, 4, 5, 6, 7, and 8)

A quick look through the results on the survival reveals that at the end of 14 days in the cold the partially hepatectomized rats showed their best performance when they were exposed to cold 48 hours after operation; in this case especially they survived much better than the sham operated or non-operated controls. On the contrary for those animals exposed to cold 3 hours after operation the partially hepatectomized survived much less than the sham operated controls, the difference in survival being 40%, but better than the non-operated controls. Those partially hepatectomized rats exposed to cold 6 hours after operation showed no significant change from the sham operated controls but much better than the absolute controls. In

the case of the rats exposed to cold (-5°C) 12 hours after operation the partially hepatectomized though their survival was about 55%, showed a better performance than both the sham operated and the non-operated controls. The animals exposed to cold 24 hours after operation showed no significant difference in survival with the sham operated controls and the survival percent in both cases was below 50%, although the partially hepatectomized group was more resistant than the sham operated up to the 10th day (65% to 30% respectively). But in the case of these animals exposed to cold 48 hours after operation, the partially hepatectomized showed a 80% survival which is 30% better than the sham operated and much better than the non-operated controls. The survival of these animals exposed to cold 72 hours after operation is almost a duplication of the results we obtained for animals exposed to cold 6 hours after operation. It seemed that even sham operation increased the resistance to cold.

b) Average loss in body weight (Table II)

The average loss in body weight is given in Table II. There seemed to be a general trend toward a relationship between percent of survival and average loss in body weight, especially for the partially hepa-

tectomized and sham operated rats exposed to cold 3, 6, 12, 24 hours after operation. Maximum loss in body weight occured on the 10th day. On the other hand, the partially hepatectomized and the sham operated controls exposed to cold 48 and 72 hours after operation lost weight steadily. The non-operated controls also lost weight steadily.

Close examination of these results reveal that the resistance of animals to cold is affected somehow by different phases of restorative activity. In our attempt to find out a possible explanation, we thought at first that increased metabolism associated with restorative activity might account for better survival observed in the partially hepatectomized rats exposed to cold (-5°C.) 48 hours after operation. The oxygen consumption determined using Depocas' et al method (54) and corrected to STP of non fasted partially hepatectomized rats in the periods of 24, 48 and 72 hours following operation showed no significant changes in $QO_2$ at the temperature of 28-30°C. This might have been due to the lack of response of the other tissues as observed by Weiss (47) and also due to the great turnover the liver was known to possess (9).

Table IV

$QO_2$ of partially hepatectomized and sham operated controls, in ml/hr/B.W.$^{0.5}$

| | Hours after operation | | | |
|---|---|---|---|---|
| | initial | 24 | 48 | 72 |
| Partial. hepat. | 29.42±1.47 | 23.20±1.70 | 24.81±0.91 | 24.32±0.68 |
| Sham operated | 28.32±2.42 | 22.66±0.90 | 29.66±2.63 | 27.42±1.17 |

On the other hand this does not eliminate the possibility that there might be an increased metabolism of the liver associated with restorative activity after cold exposure. You et al (49) have reported increased metabolic activity of visceral tissue in cold exposed animals. Weiss (48) has labelled the liver as a target of increased metabolism in cold exposed rats. It is also known that restoration activity is at its peak on the third day after operation (8). Consequently as the partially hepatectomized rats exposed to cold 48 hours after operation were actually going into the period of maximum restorative activity, it is probable that the restorative activity might have been involved possibly as a "metabolism-stimulating" (13) factor. However the contribution of the liver in overall heat production in cold exposed rat is very little.

Moreover, the results point out that the survival of the partially hepatectomized rats exposed to cold varies according to the time elapsed between partial hepatectomy and the beginning of exposure. The report of Desmarais et al (11) about the effect of partial hepatectomy on the resistance of the non-adapted rats and the observation of Weiss et al (13) that partial hepatectomy has no effect on resistance of rats exposed to cold seemed apparently contradictory. Although the severity of cold used by both investigators was not the same (Desmarais -2°C. and Weiss +5°C.) yet their different observations could be accounted for to a certain extent by the time elapsed before they exposed their operated rats to cold. Desmarais et al (11) exposed the operated rats 3 to 4 hours after operation while Weiss et al exposed their animals 24 hours after operation when the restoration of liver had already started, for a period of 3 weeks.

## TABLE II

### CHANGE IN BODY WT. OF PARTIALLY HEPATECTOMIZED (P.H.) SHAM OPERATED (S.O.) AND NON-OPERATED (C.) RATS EXPOSED TO COLD -5°C.

| Hrs.After Operation | Body Wt.at the Moment of cold Exposure** | | Average loss in Body Wt. in the Cold After | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 5 days | | 10 days | | 13 days | |
| | PH | SO | PH | SO | PH | SO | PH | SO |
| 3 | 205±4* | 210±2 | -15.3 | -13.5 | -11.3 | -7.6 | +8.8 | +4.0 |
| 6 | 206±3 | 210±3 | -11.3 | -12.6 | -10.3 | -17.1 | +6.5 | +6.4 |
| 12 | 203±2 | 211±4 | +0.6 | - 4.8 | -13.4 | -27.5 | -8.4 | -11.0 |
| 24 | 195±2 | 218±4 | -0.4 | -16.7 | - 1.1 | -26.7 | -9.2 | -18.7 |
| 48 | 267±5 | 265±7 | -8.3 | -20.6 | -14.9 | -39.0 | -17.3 | -43.4 |
| 72 | 206±7 | 218±4 | -5.1 | -12.3 | -10.6 | - 9.3 | -11.0 | -17.8 |
| | | | | C=13.8 | | C=-35.0 | | C=-37 |

* standard deviation

** body wt. of non-operated control (C) = 233±7grs.

## TABLE III

### SURVIVAL OF PARTIALLY HEPATECTOMIZED (P.H.) SHAM OPERATED (S.O.) AND NON-OPERATED RATS EXPOSED TO COLD -5°C.

| Hrs.after Operation | No. of rats used* | | % of Survivals in the Cold After | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 5 days | | 10 days | | 14 days | |
| | PH | SO | PH | SO | PH | SO | PH | SO |
| 3 | 10 | 10 | 70 | 100 | 40 | 80 | 40 | 80 |
| 6 | 10 | 10 | 70 | 90 | 60 | 70 | 50 | 40 |
| 12 | 20 | 10 | 80 | 50 | 55 | 30 | 50 | 20 |
| 24 | 20 | 10 | 70 | 80 | 65 | 30 | 40 | 30 |
| 48 | 20 | 10 | 90 | 80 | 80 | 60 | 80 | 50 |
| 72 | 20 | 10 | 80 | 80 | 55 | 60 | 55 | 60 |
| | | | | C=60 | | C=20 | | C=10 |

* non-operated control = 20 rats.

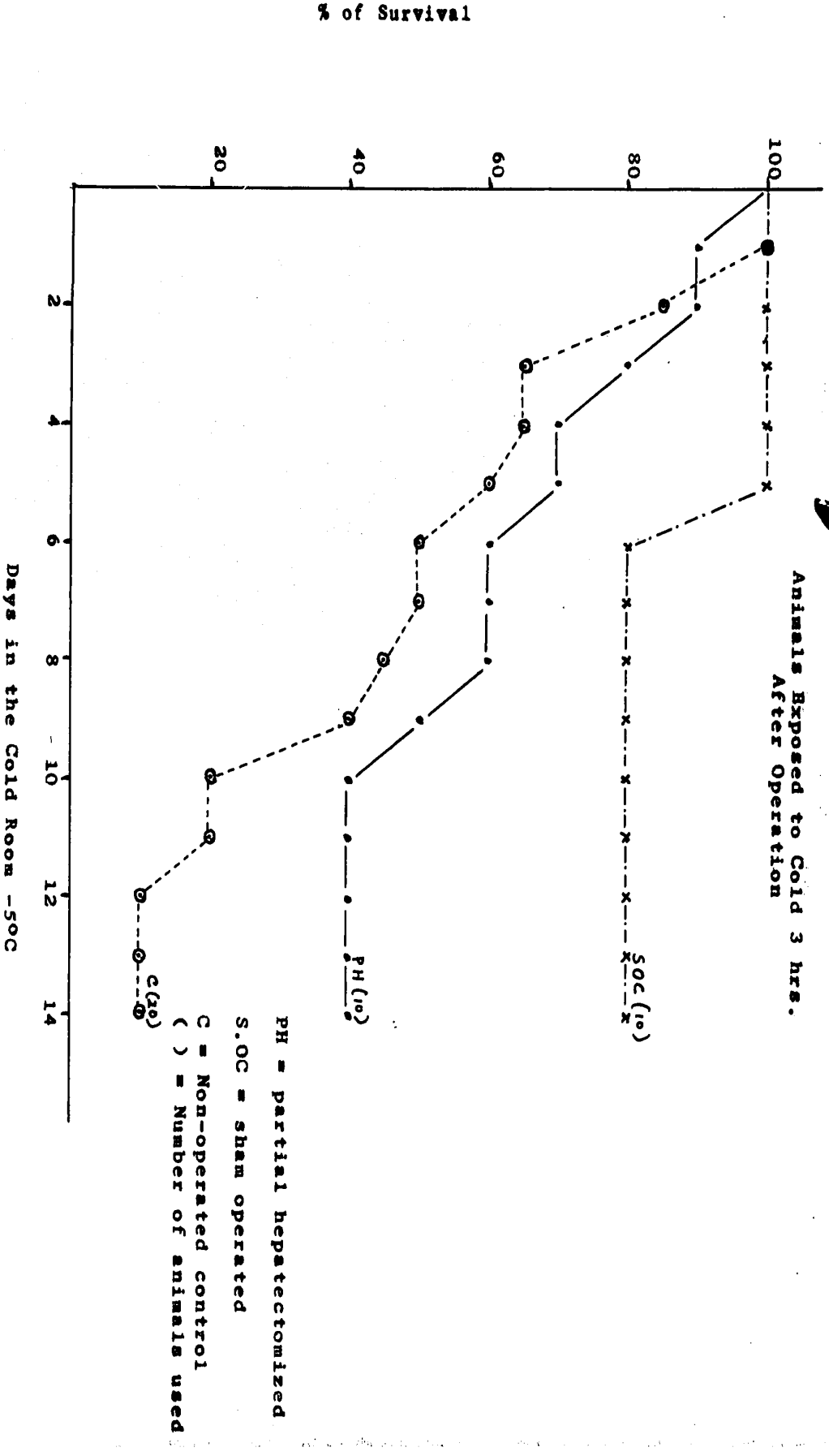

% of Survival
100
80
60
40
20
2
4
6
8
10
12
14
Days in the Cold Room -5°C
Animals Exposed to Cold 3 hrs.
After Operation
PH (10)
S.OC (10)
C(20)
PH = partial hepatectomized
S.OC = sham operated
C = Non-operated control
( ) = Number of animals used

Fig. 3

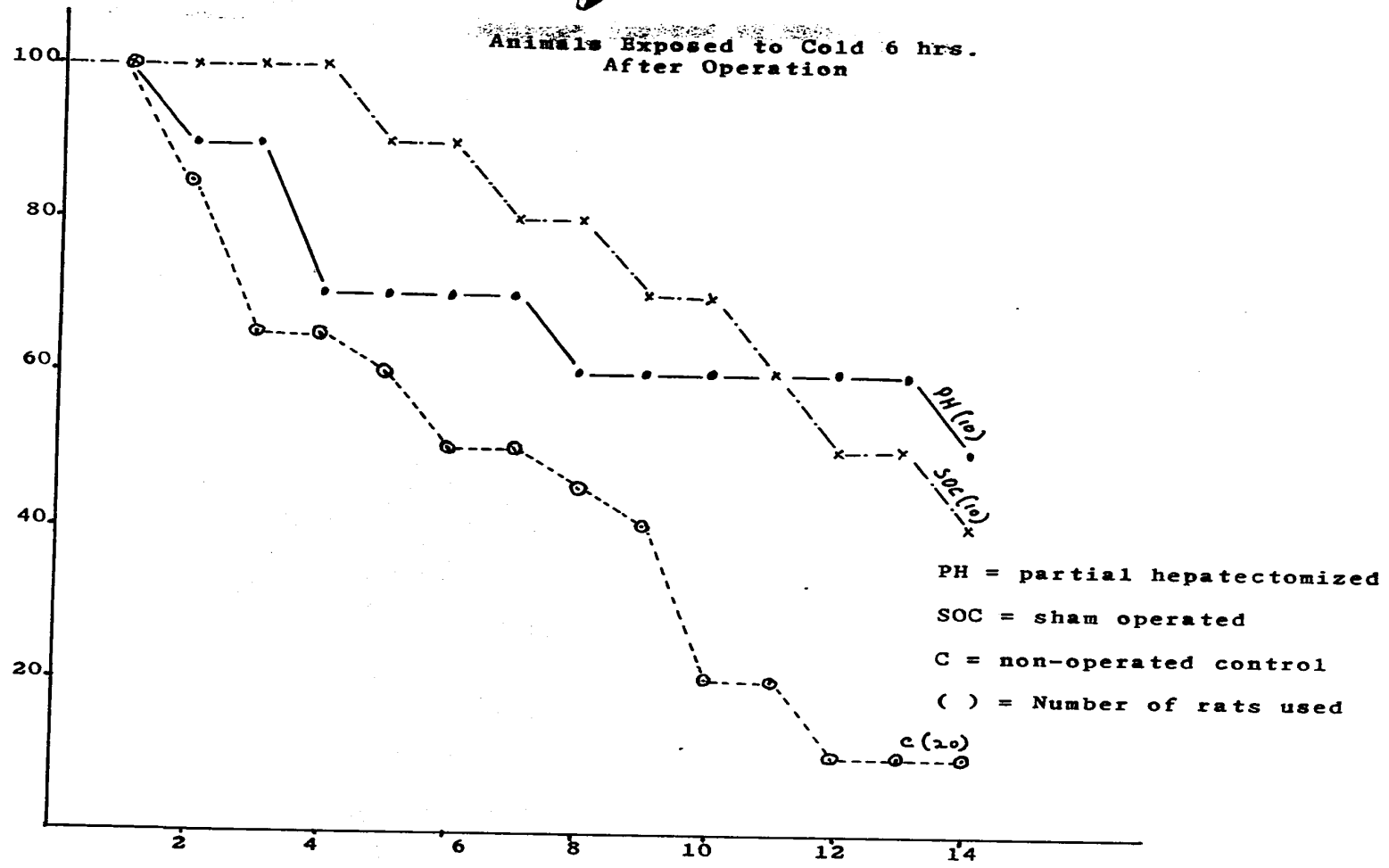

Animals Exposed to Cold 6 hrs.
After Operation
% of Survival
100
80
60
40
20
2
4
6
8
10
12
14
PH(10)
SOC(10)
C(20)
PH = partial hepatectomized
SOC = sham operated
C = non-operated control
( ) = Number of rats used
Days in the Cold Room -5°C

Fig. 4

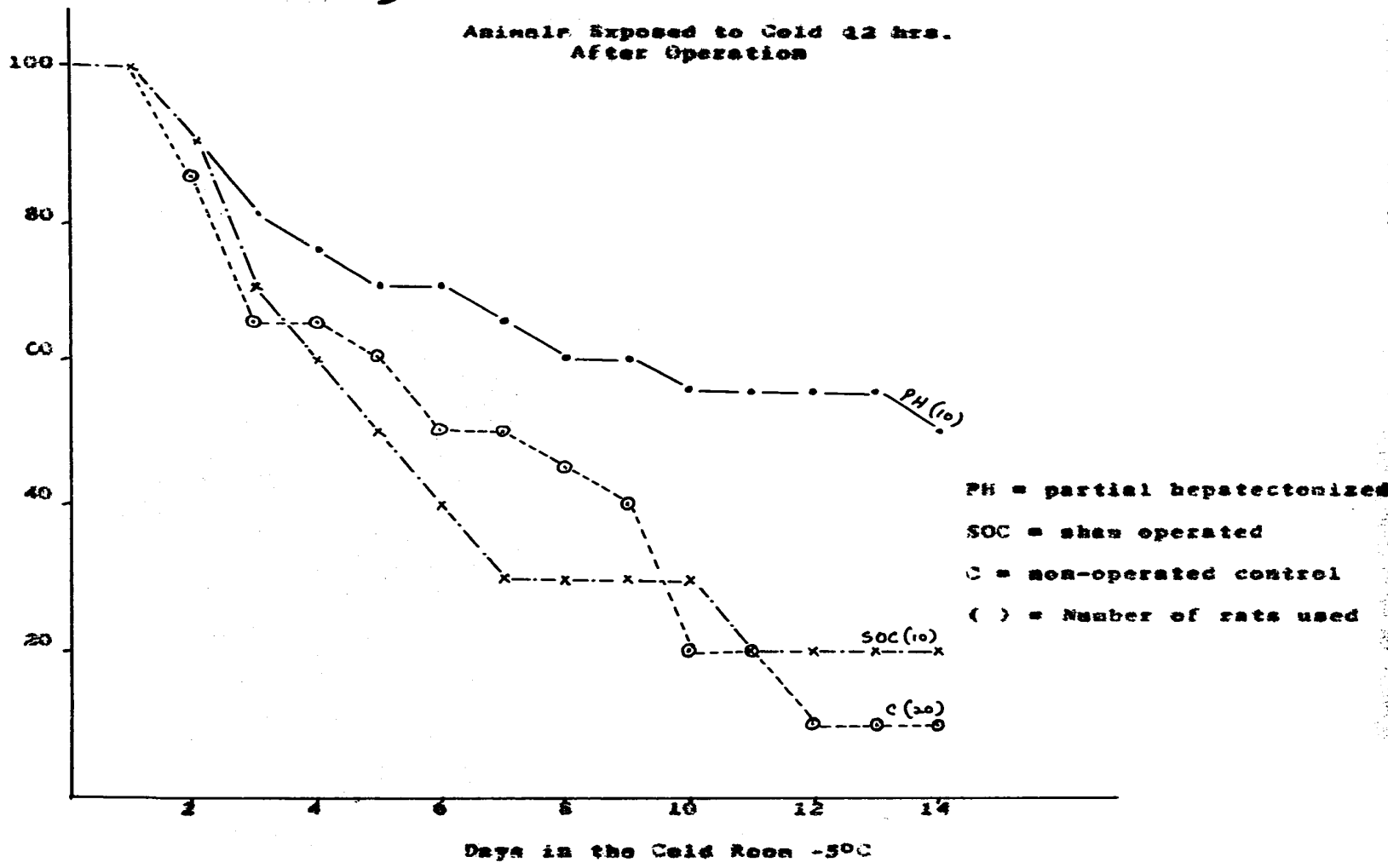
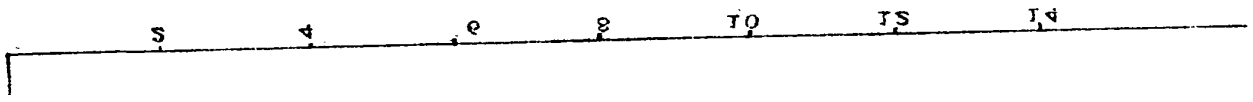

Animals Exposed to Cold 12 hrs.
After Operation
% of Survival
100
80
60
40
20
2
4
6
8
10
12
14
PH(10)
SOC(10)
C(20)
PH = partial hepatectonized
SOC = sham operated
C = non-operated control
( ) = Number of rats used
Days in the Cold Room -5°C

Fig. 5



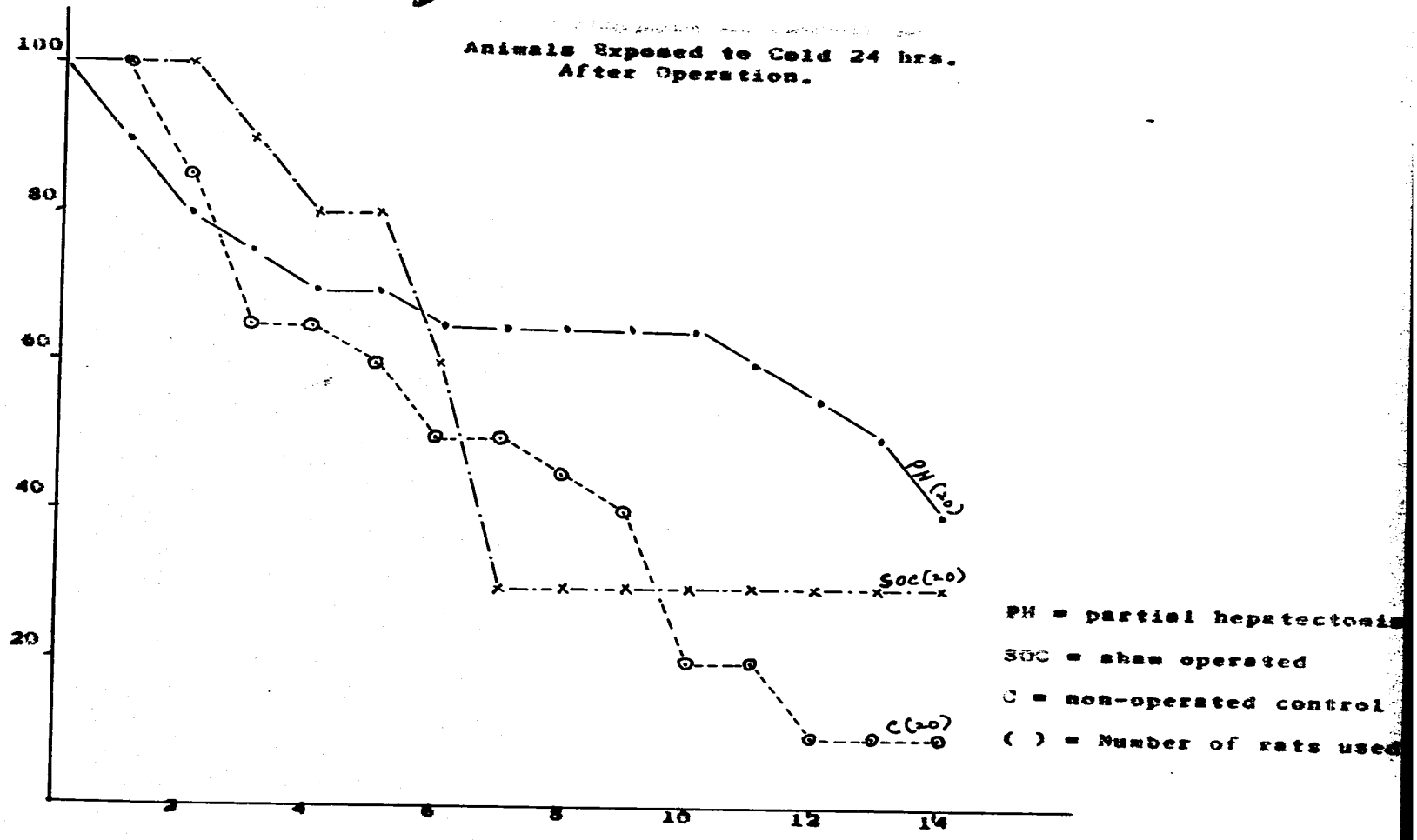

Animals Exposed to Cold 24 hrs.
After Operation.
% of Survival
100
80
60
40
20
2
4
6
8
10
12
14
PH(20)
SOC(20)
C(20)
PH = partial hepatectomi
SOC = sham operated
C = non-operated control
( ) = Number of rats used
Days in the Cold Room -5°C

Fig. 6

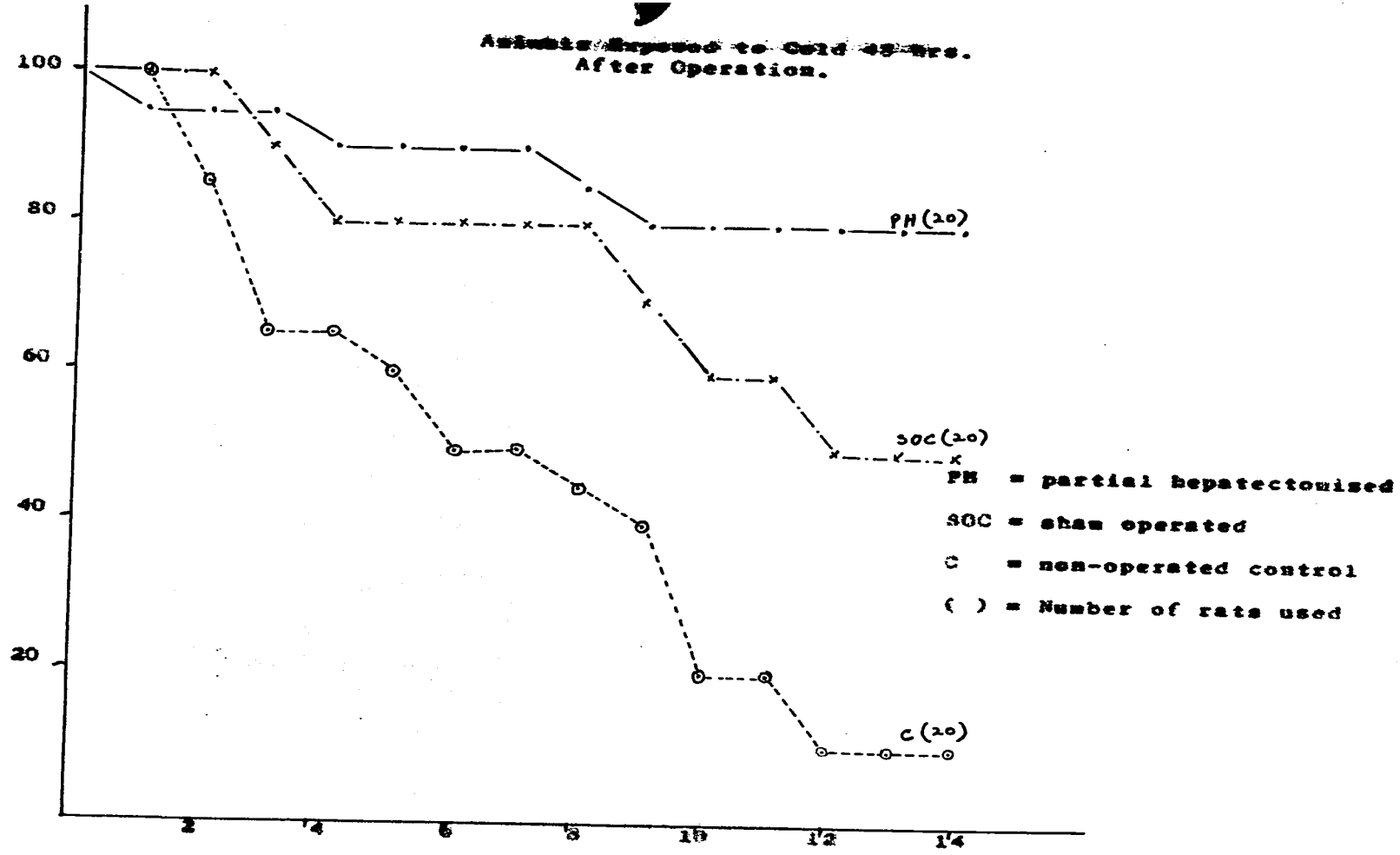

Animals Exposed to Cold 48 Hrs.
After Operation.
% of Survival
100
80
60
40
20
PH(20)
SOC(20)
C(20)
PH = partial hepatectomised
SOC = sham operated
C = non-operated control
( ) = Number of rats used
2
4
6
8
10
12
14
Days in the Cold Room -5°C

Fig. 7

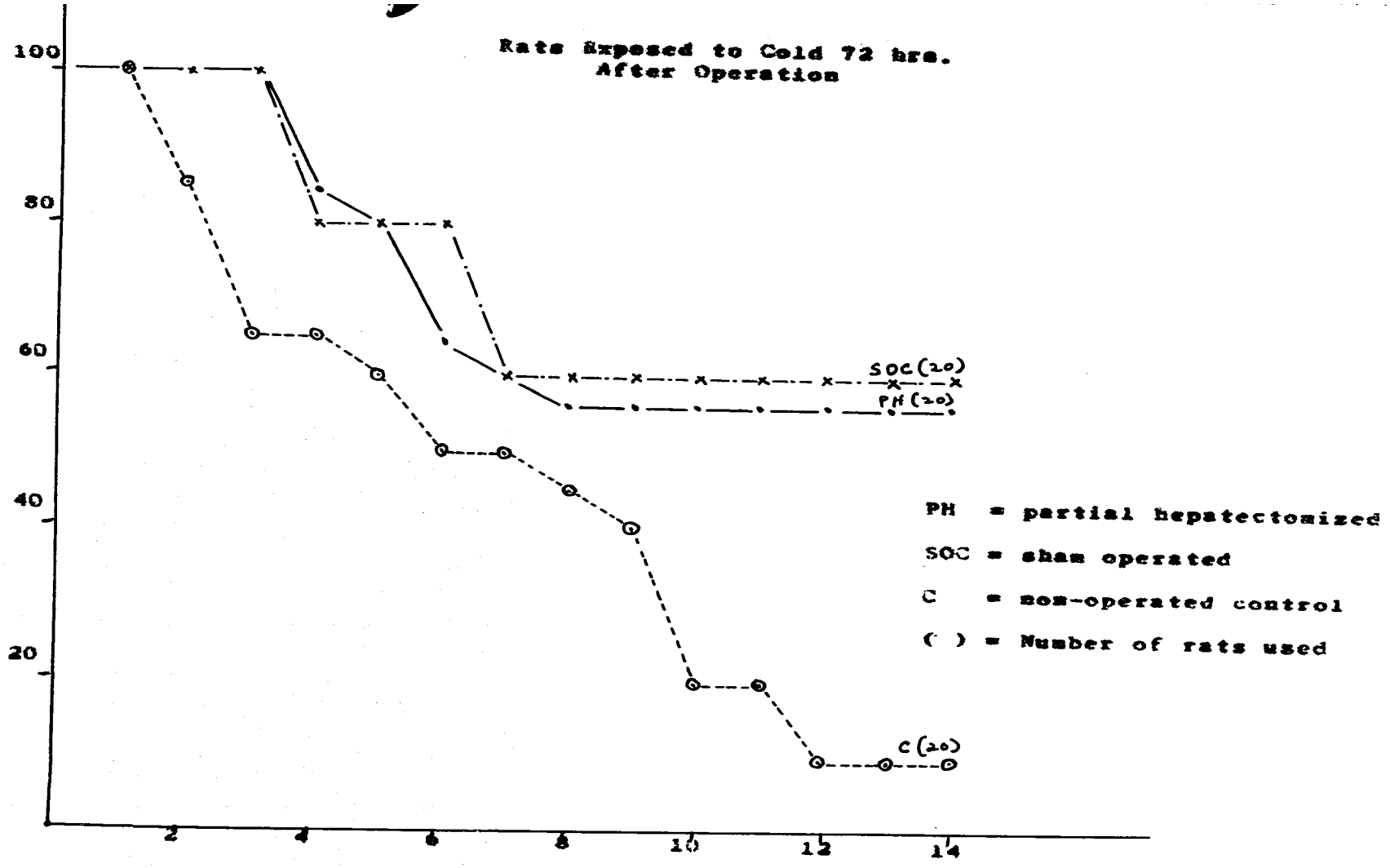

Rats Exposed to Cold 72 hrs.
After Operation
% of Survival
100
80
60
40
20
2
4
6
8
10
12
14
SOC(20)
PH(20)
C(20)
PH = partial hepatectomized
SOC = sham operated
C = non-operated control
( ) = Number of rats used
Days in the Cold Room -5°C

Fig. 8

## Third series of experiments

### Introduction

In our previous two series of experiments we have reported how the vitamin C content of the restoring liver and the resistance of animals to cold were affected by the restorative activity, of the liver, noted by Higgins et al (3). It was logical, therefore, to see if the close relation that was found to exist between the vitamin C content and the restorative activity at room temperature held true in the case of non-adapted and adapted animals exposed to cold (-5°C.). The following series of experiments were designed to this end.

### Materials and Methods

About 340 male albino rats of Wistar strain 8 weeks old and weighing 150-200 grams were divided into two groups according to their weights.

#### Group I

Two-hundred-and-thirty-four rats weighing 150-180 grams were kept at room temperature (20°±2°C) for a month before they underwent operation. After a month, their weights were between 250 and 350 grams

approximately. Ninety were partially hepatectomized, 72 were sham operated and 72 served as non-operated controls. These were divided into 9 subgroups. Each subgroup consisted of:-

a) 9-11 (most of the time 10) partially hepatectomized rats,

b) 7-9 (most of the time 8) sham operated rats, and

c) 7-9 (most of the time 8) non-operated controls.

These groups were exposed to cold 3 hours after operation and were sacrificed 12, 24, 48, 72, 96, 120, 168, 216 and 264 hours after operation respectively. The periods of 144, 192 and 240 were skipped as we did not see any significant change in vitamin C during these periods at room temperature. The time of autopsy in each case included the 3 hours elapsed before the rats were put in the cold after operation.

Group II

Two-hundred-and-seventy male albino rats weighing between 200 and 220 grams were kept in the cold room (20±2°C.) for a month, the same duration of

time as the animals of Group I were kept at room temperature. Sellers et al (50) have reported that the degree of "acclimatization" appears to be maximum between the period of 4 to 6 weeks after exposure to cold. Consequently the animals that survived the cold temperature 2°±2°C. for a month and showed increased in body weight are referred to as "adapted" animals. During the experimentation we had to discard many animals which did not fit the above requirements, especially steady increase in body weight. The rats of Group II underwent the same experimental protocol and at the same time as those of Group I.

Both at operation time and at sacrifice time, in the two groups, the removed liver was weighed and used for the determination of vitamin C, and at autopsy it was used for determination of the restoration percent [illegible].

Results and Discussion

Before we give highlights of the results we would like to bring into attention two points. First, as we have already stated, all the animals were exposed to cold 3 hours after operation (time considered

enough for recovery from ether anesthesia). Therefore, time in the cold room is equal to time after operation minus 3 hours in each case. Every group was followed through till the 11th day except in the cases where the survival dropped below 50% before the end of the experiment.

A. Vitamin C Content

i) Non-adapted animals

The initial vitamin C concentration of the liver of the non-adapted rats at the moment of surgery was 0.27±0.02 mg/gm of wet weight of the liver. The results are given in Tables V, VII, VIII and Figures 9 and 13. Twelve hours after partial hepatectomy the vitamin C concentration experienced a drop. Further drop in vitamin C concentration was observed 24 hours after partial hepatectomy. Then after 48 hours the level of vitamin C was found to be almost the same as at 12 hours after operation. At the 72nd hour the vitamin C seemed to approach the normal content but still it is significantly below ($P < 0.001$) the initial level. Ninety-six hours after operation the vitamin C concentration was almost the same as 72 hours after operation. At the 120th hour

after operation a drop in vitamin C was noted almost to the same level as 48 hours after operation. The 168th hours after operation, the vitamin C concentration seemed to rise up again. The data after this time though tabulated were not considered as the survival of animals was below 50%.

The vitamin C content of the liver of sham operated controls showed a gradual drop till the 72nd hour after which they maintained almost the same level of vitamin C except at 168 hours after operation when the vitamin C level almost corresponded to the initial level. (Fig. 9, and Table VII). On the other hand, the non-operated control showed a sudden rise in vitamin C content 9 hours after being in the cold; then the level of vitamin C dropped to the level of 0.80 mg/gm of wet weight of liver. (Fig. 9 and Table VIII). This level was almost maintained throughout the experiment with slight variations.

ii) Adapted Animals

The adapted partially hepatectomized rats showed no significant change in vitamin C concentration 12 hours after operation when compared with the initial vitamin C level at the moment of surgery

namely 0.25±0.02 mg/gm of wet weight and of liver. Twenty-four hours after, the level of vitamin C suddenly dropped to a level of 0.15 mg/gm of wet weight (P 0.001). (Fig. 10, Table VI). Then a rise in vitamin C concentration was observed 48 hours after operation followed by a slight rise 72 and 96 hours after operation, but still significantly below the initial vitamin C concentration. The period from 120 hours until 216 hours showed no significant variations and the level of vitamin C was maintained just slightly below the one noted for 96 hours after operation.

The adapted sham operated controls showed a sudden rise 12 hours after operation followed by return to the initial level 24 hours after operation. (Fig. 10, Table VII). The period between 48 and 72 hours after operation exhibited a gradual decrease in vitamin C followed by a gradual rise from the period of 96 hours after operation until 216th hour after operation. The data for 240th hour were discarded as the survival was below 30%.

The adapted non-operated controls showed a sudden but not so great a rise as the sham operated

after 9 hours in the cold. (Fig. 10, Table VIII). Twenty-one hours in the cold room brought the level of vitamin C to the initial level. They showed a drop in vitamin C concentration after 45 hours in the cold, followed by an increase level on the 69th hour after cold exposure. The period from the 69th till the 117th hour in the cold indicated a gradual decrease in vitamin C concentration followed by a sudden rise after 165 hours in the cold, then dropping almost to the level of vitamin C observed after 117 hours. The data after this were discarded as the survival was below 50%.

b) Restoration (Tables V and VI, Fig. 11)

Both the adapted and the non-adapted rats exposed to cold showed restoration. (Figure 11, and Tables V, VI). Maximum restoration occured between 48 and 120 hours after operation in both cases. The adapted rats showed a higher restoration than the non-adapted rats; the differences in restoration between the two groups being 14.7%, 29.5%, 30.1% and 13.1% for the post-operative periods of 48, 72, 96 and 120 hours respectively. Figure 11 illustrates this.

The results indicate that the vitamin concentration of both adapted and non-adapted rats followed almost the same general pattern we have observed in the first series of experiments. The vitamin C concentration of the restoring liver of the adapted rats though lower initially was more than of the non-adapted rats during the restorative periods in the cold. The results point out that the variation of vitamin C concentration still goes hand in hand with the restorative activity as observed by Higgins et al (8).

The restoration of the liver in the adapted rats was found to be faster than in the non-adapted ones (Fig. 11); in both cases, however, the restoration in the cold was lower than the one observed at room temperature as Fig. 14 indicates especially after 5 days of operation. This particular results is in direct disagreement with the one of Weiss (32) who reported an enhanced restoration in the cold exposed partially hepatectomized rats but corroborates his observation with milder cold (+5°C) (13).

TABLE V

Vitamin C Content and Restoration of Liver of non-adapted rats exposed to cold -5°C

| AT OPERATION | | | | AT SACRIFICE | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| No. of rats used | Body wt. grs. | Wt. of liver removed grs. | Est. wt. of liver remaining grs. | No. of rats | Body wt. grs. | LIVER REMAINING Wet wt. grs. | Restoration % | L-ASCORBIC ACID mg/gm of wet weight | (P) | Hours after operation |
| 10 | 326±2* | 6.40±.38 | 3.44±.17 | 10 | 296±10 | 4.98±.23 | 45.9±8.8 | 0.20±0.04** | 0.001 | 12 |
| 10 | 302±8 | 6.69±.44 | 3.66±.19 | 9 | 273±8 | 4.96±.18 | 37.0±4.3 | 0.15±0.01 | 0.001 | 24 |
| 9 | 300±9 | 7.73±.47 | 4.16±.26 | 9 | 266±8 | 6.47±.29 | 57.9±6.5 | 0.18±0.01 | 0.001 | 48 |
| 11 | 323±3 | 8.05±.39 | 4.33±.21 | 9 | 280±10 | 7.84±.38 | 82.4±8.6 | 0.20±0.00 | 0.001 | 72 |
| 9 | 302±10 | 7.71±.44 | 4.18±.24 | 9 | 268±9 | 9.05±.51 | 106.3±13.8 | 0.19±0.01 | 0.001 | 96 |
| 9 | 290±11 | 7.08±.47 | 3.69±.31 | 8 | 266±14 | 9.20±.59 | 154.1±12.4 | 0.16±0.02 | 0.001 | 120 |
| 10 | 311±11 | 7.70±.23 | 4.14±.13 | 9 | 272±6 | 8.81±.16 | 113.8±6.9 | 0.20±0.01 | 0.001 | 168 |
| 10 | 340±10 | 8.03±.65 | 4.32±.35 | 4 | 302±12 | 10.21±.71 | 137.9±13.7 | 0.18±0.01 | 0.001 | 216 |
| 6 | 311±4 | 7.25±.38 | 3.90±.21 | 4 | 266±9 | 9.45±.09 | 144.4±12.8 | 0.17±0.01 | 0.001 | 264 |

* standard deviation
** vitamin C concentration at time 0 = 0.27±0.02 mg/gm of wet wt.
P level of significance.

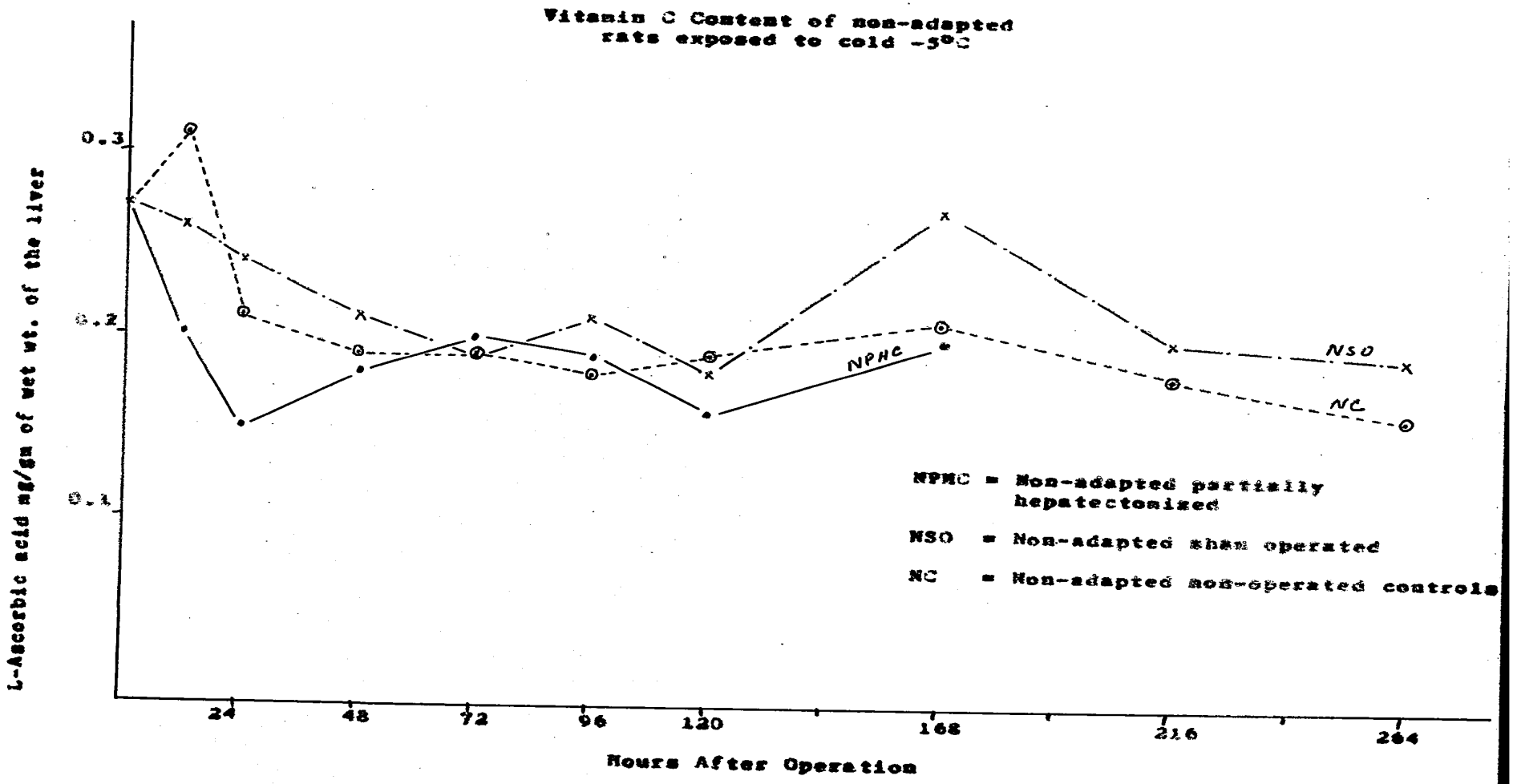

Vitamin C Content of non-adapted rats exposed to cold -5°C
L-Ascorbic acid mg/gm of wet wt. of the liver
0.3
0.2
0.1
24
48
72
96
120
168
216
264
Hours After Operation
NPHC
NSO
NC
NPHC = Non-adapted partially hepatectomized
NSO = Non-adapted sham operated
NC = Non-adapted non-operated controls

Fig. 9

TABLE VI

Vitamin C Content and Restoration of Liver of adapted rats exposed to cold -5°C

| AT OPERATION | | | | AT SACRIFICE | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| No. of rats used | Body wt. grs. | Wt. of liver removed grs. | Est. wt. of liver remaining grs. | No. of rats | Body wt. grs. | LIVER REMAINING Wet wt. grs. | Restoration | L-ASCORBIC ACID mg/gm of wet weight | (P) | Hours after operation |
| 9 | 261±7* | 5.94±.25 | 3.30±.13 | 9 | 242±6 | 4.16±.14 | 31.2±4.7 | 0.24±0.02** | 0.75 | 12 |
| 10 | 251±8 | 5.36±.24 | 2.83±.13 | 10 | 254±7 | 4.44±.20 | 56.9±7.4 | 0.13±0.01 | 0.001 | 24 |
| 10 | 229±13 | 6.37±.43 | 3.43±.23 | 10 | 219±13 | 5.69±.20 | 72.6±6.8 | 0.19±0.02 | 0.01 | 48 |
| 9 | 244±14 | 6.70±.39 | 3.60±.21 | 7 | 225±11 | 7.57±.38 | 111.9±7.3 | 0.21±0.01 | 0.01 | 72 |
| 10 | 253±14 | 6.96±.36 | 3.76±.21 | 9 | 253±11 | 8.83±.44 | 136.4±6.8 | 0.21±0.02 | 0.05 | 96 |
| 9 | 265±6 | 6.90±.32 | 3.34±.40 | 9 | 250±9 | 8.84±.32 | 167.2±4.9 | 0.19±0.01 | 0.001 | 120 |
| 10 | 261±6 | 6.34±.22 | 3.41±.11 | 7 | 215±6 | 7.74±.26 | 127.9±5.9 | 0.21±0.01 | 0.025 | 168 |
| 9 | 274±11 | 6.96±.25 | 3.74±.13 | 7 | 299±10 | 9.35±.20 | 150.9±7.9 | 0.20±0.00 | 0.001 | 216 |
| 9 | 231±7 | 5.33±.74 | 2.87±.40 | 9 | 231±17 | 5.26±.39 | 165.5±42.0 | 0.18±0.04 | 0.25 | 264 |

* standard deviation

** Vitamin C concentration at time 0 = 0.22±0

P Level of significance

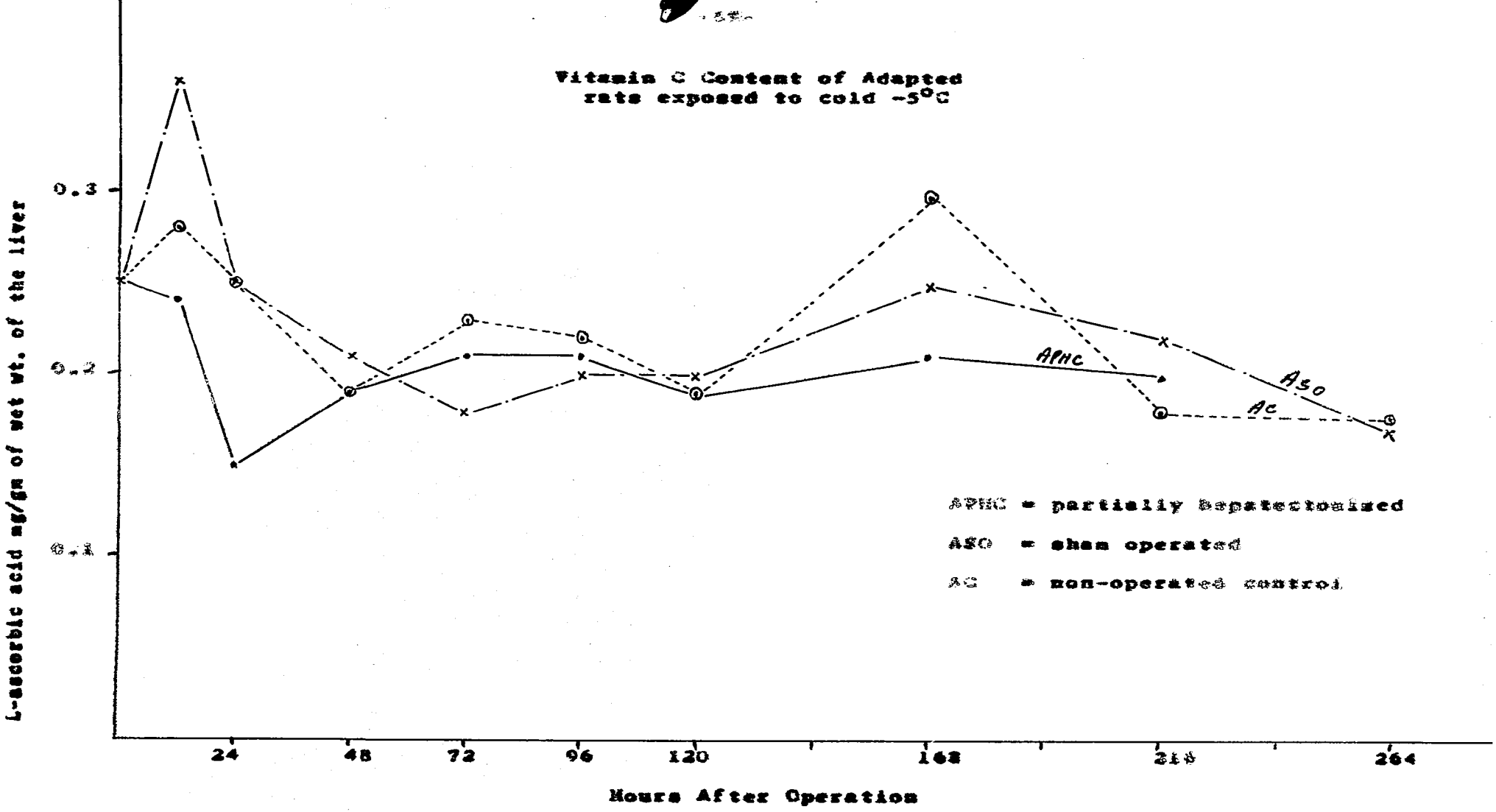

Vitamin C Content of Adapted rats exposed to cold -5°C
L-ascorbic acid mg/gm of wet wt. of the liver
0.3
0.2
0.1
24
48
72
96
120
168
216
264
Hours After Operation
APHC
ASO
AC
APHC = partially hepatectomized
ASO = sham operated
AC = non-operated control

Fig. 10

TABLE VII

Vitamin C Content of Non-adapted (NSO) and Adapted (ASO) sham operated rats exposed to cold -5°C

| | No.of rats used | Initial body wt. | AT SACRIFICE No.of rats | Body wt. gms. | LIVER * wt. gm. | L-ASCORBIC ACID Mg/gm of wet wt. | (P) | Hours after operation |
|---|---|---|---|---|---|---|---|---|
| NON-ADAPTED | 9 | 321±10* | 9 | 314±11 | 12.26±0.56 | 0.26±0.03 | 0.70 (P) | 12 |
| | 9 | 292±9 | 9 | 280±10 | 10.18±0.42 | 0.24±0.01 | .10 | 24 |
| | 8 | 282±6 | 8 | 269±7 | 9.83±0.41 | 0.21±0.01 | .10 | 48 |
| | 7 | 287±13 | 7 | 270±12 | 9.52±0.31 | 0.19±0.01 | .001 | 72 |
| | 7 | 301±8 | 7 | 275±7 | 9.44±0.42 | 0.21±0.02 | .001 | 96 |
| | 8 | 290±8 | 7 | 266±8 | 10.41±0.60 | 0.18±0.02 | .020 | 120 |
| | 7 | 292±11 | 4 | 272±17 | 9.42±0.74 | 0.27±0.01 | | 168 |
| | 7 | 357±13 | 6 | 322±10 | 11.69±0.53 | 0.20±0.01 | .001 | 216 |
| | 7 | 306±7 | 4 | 259±2 | 10.61±1.04 | 0.19±0.01 | .001 | 264 |
| ADAPTED | 9 | 257±7 | 9 | 252±6 | 9.32±0.46 | 0.36±0.03 | .005 | 12 |
| | 8 | 275±11 | 8 | 267±8 | 9.58±1.32 | 0.25±0.02 | | 24 |
| | 7 | 329±11 | 7 | 220±16 | 8.79±0.28 | 0.21±0.01 | .025 | 48 |
| | 6 | 249±8 | 4 | 247±4 | 10.31±0.71 | 0.18±0.02 | .001 | 72 |
| | 5 | 243±15 | 5 | 231±15 | 8.82±0.51 | 0.20±0.02 | .10 | 96 |
| | 7 | 251±9 | 6 | 245±10 | 10.36±0.61 | 0.20±0.02 | .05 | 120 |
| | 6 | 223±2 | 5 | 205±6 | 8.24±0.40 | 0.25±0.02 | | 168 |
| | 6 | 275±7 | 4 | 212±53 | 10.01±0.13 | 0.22±0.02 | .20 | 216 |
| | 7 | 239±10 | 4 | 221±2 | 9.05±1.06 | 0.17±0.01 | .001 | 264 |

* standard deviation

P level of significance

TABLE VIII

THE EFFECT OF COLD ON THE VITAMIN C CONTENT
OF ADAPTED (AC) AND NON-ADAPTED (NC) NON-OPERATED CONTROLS

| | No.of rats used | Initial body wt. | No.of rats | AT SACRIFICE Body wt. gms. | LIVER wet wt. gm. | L-ASCORBIC ACID mg/gm of wet wt. | (P) | Hours in Cold |
|---|---|---|---|---|---|---|---|---|
| NON-ADAPTED | 8 | 313±15* | 8 | 297±10 | 11.57±0.52 | 0.31±0.05 | 0.40 | 9 |
| | 9 | 317±16 | 9 | 303±6 | 11.66±0.31 | 0.21±0.02 | 0.010 | 21 |
| | 8 | 288±7 | 8 | 281±8 | 11.40±0.43 | 0.19±0.01 | .001 | 45 |
| | 8 | 320±9 | 8 | 313±9 | 11.83±0.36 | 0.19±0.01 | .001 | 69 |
| | 8 | 300±11 | 8 | 283±10 | 10.61±0.38 | 0.18±0.01 | .001 | 93 |
| | 7 | 303±10 | 7 | 236±10 | 11.66±0.47 | 0.19±0.01 | .001 | 117 |
| | 7 | 320±16 | 7 | 278±15 | 11.19±0.70 | 0.21±0.01 | .001 | 165 |
| | 6 | 399±9 | 6 | 347±9 | 13.53±0.33 | 0.18±0.01 | .001 | 213 |
| | 7 | 316±9 | 5 | 271±7 | 11.81±0.75 | 0.16±0.01 | .001 | 261 |
| ADAPTED | 9 | 248±8 | 9 | 221±29 | 10.31±0.30 | 0.28±0.05 | .60 | 9 |
| | 8 | 268±8 | 8 | 268±8 | 11.46±0.31 | 0.25±0.01 | .005 | 21 |
| | 7 | 229±15 | 6 | 217±40 | 9.73±0.92 | 0.19±0.01 | .30 | 45 |
| | 6 | 243±20 | 6 | 243±20 | 10.05±0.62 | 0.23±0.04 | .20 | 69 |
| | 7 | 237±16 | 7 | 237±16 | 9.71±0.51 | 0.22±0.02 | .10 | 93 |
| | 4 | 212± | 3 | 212±14 | 8.81±0.67 | 0.19±0.04 | .20 | 117 |
| | 7 | 252±33 | 7 | 252±10 | 9.66±0.28 | 0.30±0.04 | .02 | 165 |
| | 6 | 280±39 | 6 | 280±11 | 10.92±0.77 | 0.18±0.04 | .05 | 213 |

* standard deviation

P level of significance

RESTORATION OF THE LIVER OF NON-ADAPTED (NPHC) AND ADAPTED (APHC) RATS EXPOSED TO COLD -5°C

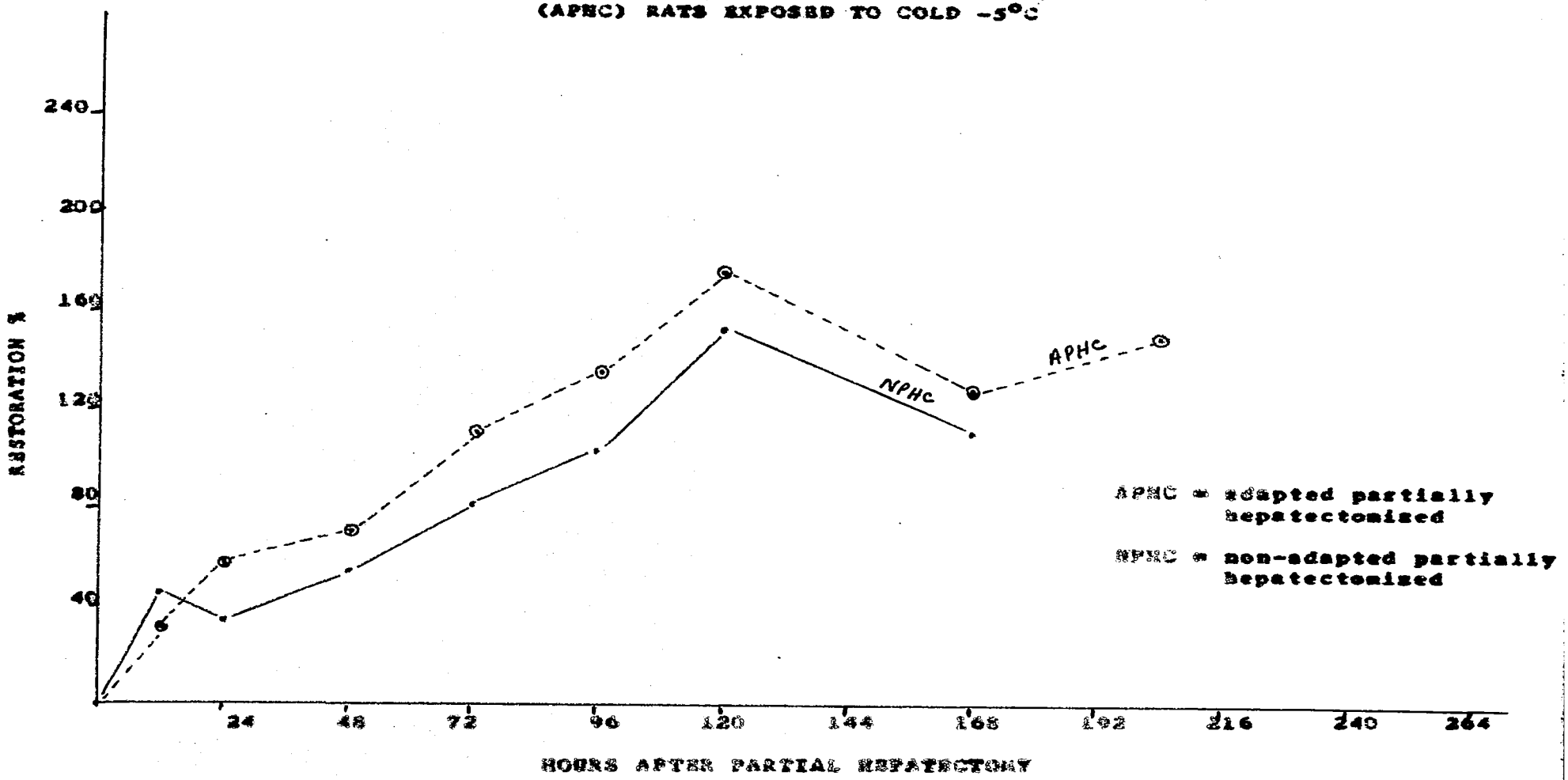



Fig. 11

L-ASCORBIC ACID CONTENT OF REGENERATING LIVER OF NON-ADAPTED (NPHC) AND OF ADAPTED (APHC) RATS EXPOSED TO COLD -5°C

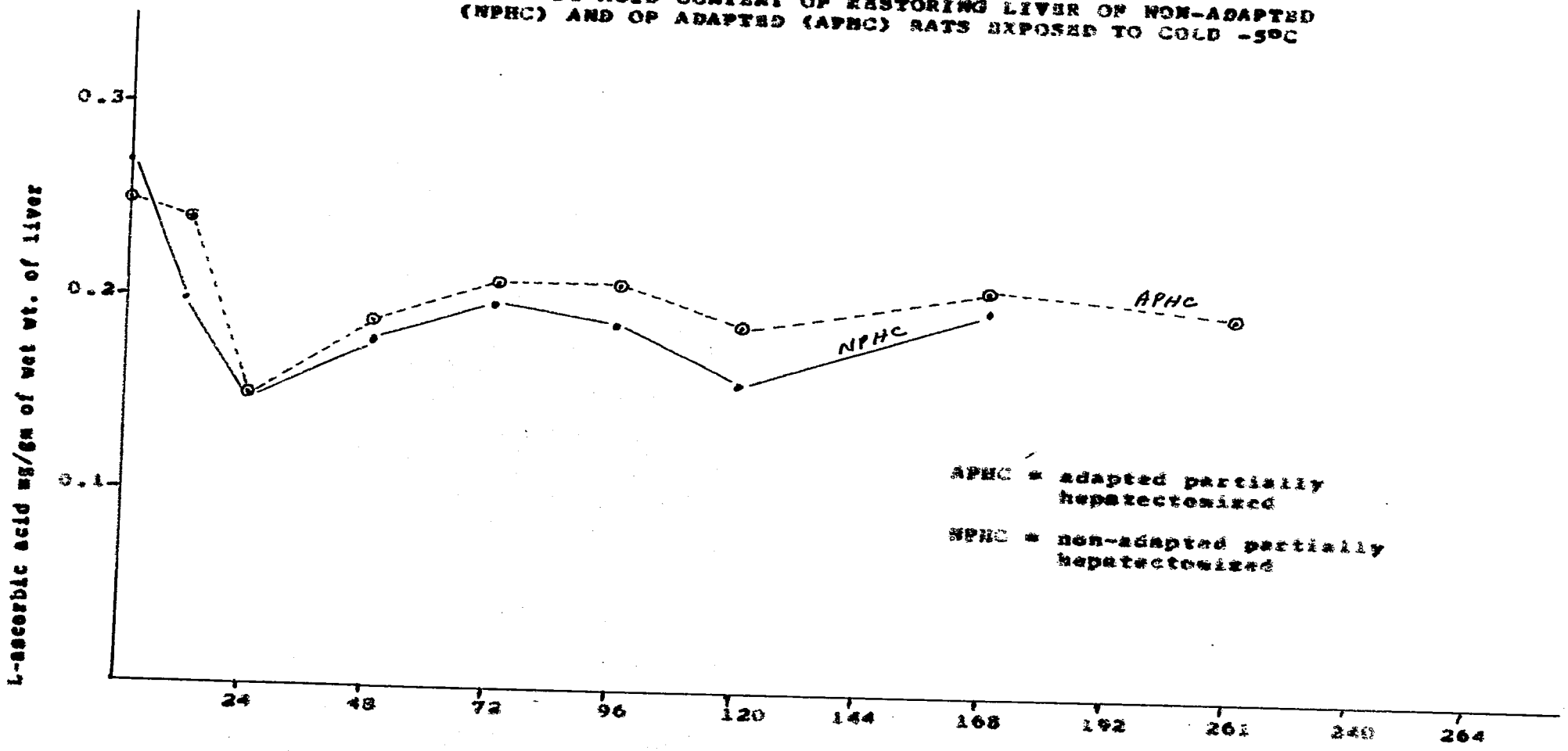



Fig-12

## GENERAL DISCUSSION

The purpose of this research was to find out if the various effects of partial hepatectomy on the resistance of animals to cold would be related to the variations in the vitamin C concentration of the tissues especially that of the liver during restoration. This hypothesis has been tested by carrying out three series of experiments. During these experiments many observations were made. Since most of the observations were noted for the first time, their bearings on the restoration of the liver of the rats after partial hepatectomy need to be brought out.

First of all, a close relation between the vitamin C concentration and the restorative activity as reported by Higgins et al (8) after partial hepatectomy was observed at room temperature (Table I and Figure 1). This relation was also found to be true, during the whole period of our experiment for the restoring liver of the adapted and non-adapted partially hepatectomized rats exposed to cold (-5°C) 3 hours after operation (Tables V and VI and Figures 9, 10, and 12). The relation of the

vitamin C concentration and the restorative activity of the restoring liver under all conditions of our experiments has been interpreted as suggestive of a possible role of vitamin C in the restoration of the liver possibly mediated through its strong oxidation-reduction reactions.

Due to technical error, we were unable to observe variations in dry wt during restoration noted by Higgins et al (8) and Dugal et al (55).

The maximum restoration occured between the 2nd and 4th days after partial hepatectomy both at room temperature and in the cold (~5°C) (Fig. 14). This was quite in agreement with Higgins et al (8) though they found maximum restoration between the 3rd and 4th day. They calculated the restoration in terms of 100 gm of body weight while we calculated it in terms of the remaining liver after surgery, employing Weiss' method (13). The variations in body weight, especially in the cold, did not justify the expression of restoration in terms of body weight. As indicated in Tables IV and V, both the non-adapted and adapted partially hepatectomised rats did not even

regain their initial body weight during the whole duration of the experiments and this was also observed by Weiss (13) with milder cold (+5°C). It was evident, therefore, that if the restoration was expressed in terms of body weight, the true picture of the restoration would not be given. On the other hand, the partially hepatectomized rats at room temperature gradually attained their initial body weight about the 96th hour after operation and thereafter showed a steady increase in body weight (Table I).

Chronic cold exposure appeared not to affect the restoration of the liver of both the adapted and the non-adapted rats up to 5 days after operation (Fig. 14) and this has already been reported by Weiss et al (13) with mild cold (+5°C). But after the period of 5 days the restoration seemed to be affected by chronic cold exposure and the retarding effect of chronic cold appeared to be lessen by cold adaptation.

Rats exposed to cold (-5°C) after different post-operative periods (3, 6, 12, 24, 48 and 72 hours after operation) showed variations in their resistance (Fig. 3, 4, 5, 6, 7 and 8). The survival was very low for those exposed to cold 3 hours after operation;

then the survival gradually increased through the post-operative periods of 6, 12, 24, 48 hours when the survival was maximum (80%), and decreased 72 hours after operation. In our attempt to explain this dramatic behaviour of partially hepatectomized rats, we thought at first that increased metabolism associated with restorative activity might account for this. There was no variation in $CO_2$, of non-fasted partially hepatectomized rats whether its determination was made 24, 48 or 72 hours after operation at temperature of 28-30°C (Table IV). On the other hand, when one considers that among other things, the ability of the rat to produce and maintain heat is very important in the resistance of rats exposed to cold, there seemed to be a possibility that partially hepatectomized rats exposed to cold (-5°C) 48 hours after operation might have a greater ability for heat production. This, of course, needs to be assessed by determining the metabolic rate of the animals at interval of time after being exposed to cold.

There seems to be a relation between the restorative activity of the liver and the resistance shown by rats exposed to cold. It is known that the

liver of the rat starts to restore about 24 hours after partial hepatectomy and that the peak of the restorative activity is found about the 72nd hour after operation (8). The survival of rats exposed to cold 3, 6 and 48 hours after operation might be due to the effect of cold on the restoration of the liver while the rats exposed to cold 24 hours after operation showed a better survival than the preceding animals since the liver had already started to restore but the retarding effect of cold still seemed to be there. On the other hand, in the rats exposed to cold 48 hours after operation, the restoration was well underway and it was going into the period of peak restorative activity. In this case, cold exposure apparently seemed to have no effect as the rats showed a remarkable survival. The rats exposed to cold 72 hours after operation did not survive so well. This last result is not too surprising if one admits that the maximum restorative activity does not coincide with the points shown in the graph (Fig. 2), but rather preceded them.

Furthermore, though the exact relation of the level of vitamin C of the liver and the vitamin C "pool" of the rat is not known, yet it appears that the level of vitamin C of the restoring liver might have been beneficial in these rats exposed to cold 48 hours after operation since these rats were actually going into the period of normal when the level of vitamin C approached the initial level, namely 72nd hour after operation.

So we see that the effect of partial hepatectomy on the resistance of rats to cold involves many factors. Among these various factors, the time elapsed between partial and exposure to cold is very important. Any attempt at this time to correlate the various effects of partial hepatectomy on the resistance of the rat to cold with the level of vitamin C of the restoring liver, even though there seems to be a direct relation between restorative activity and the level of vitamin C of the restoring liver, would be premature; this can only be ventured when the exact relation between the level of liver vitamin C and the vitamin C "pool" of the rat will have been assessed.

VITAMIN C CONTENT OF REGROWING LIVER IN RATS KEPT AT ROOM TEMPERATURE (PHR) AND IN NON-ADAPTED (NPHC) AND ADAPTED RATS EXPOSED TO COLD -5°C.

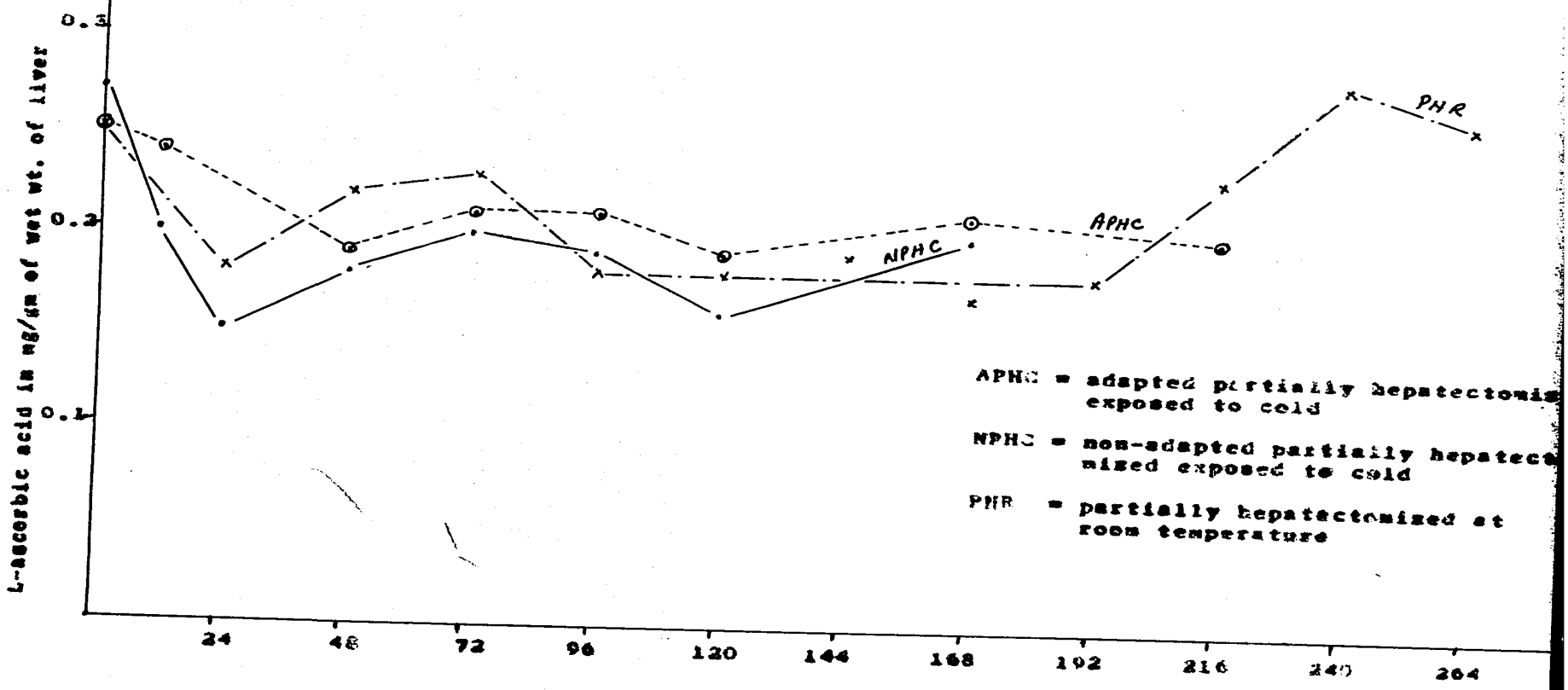



Fig. 13

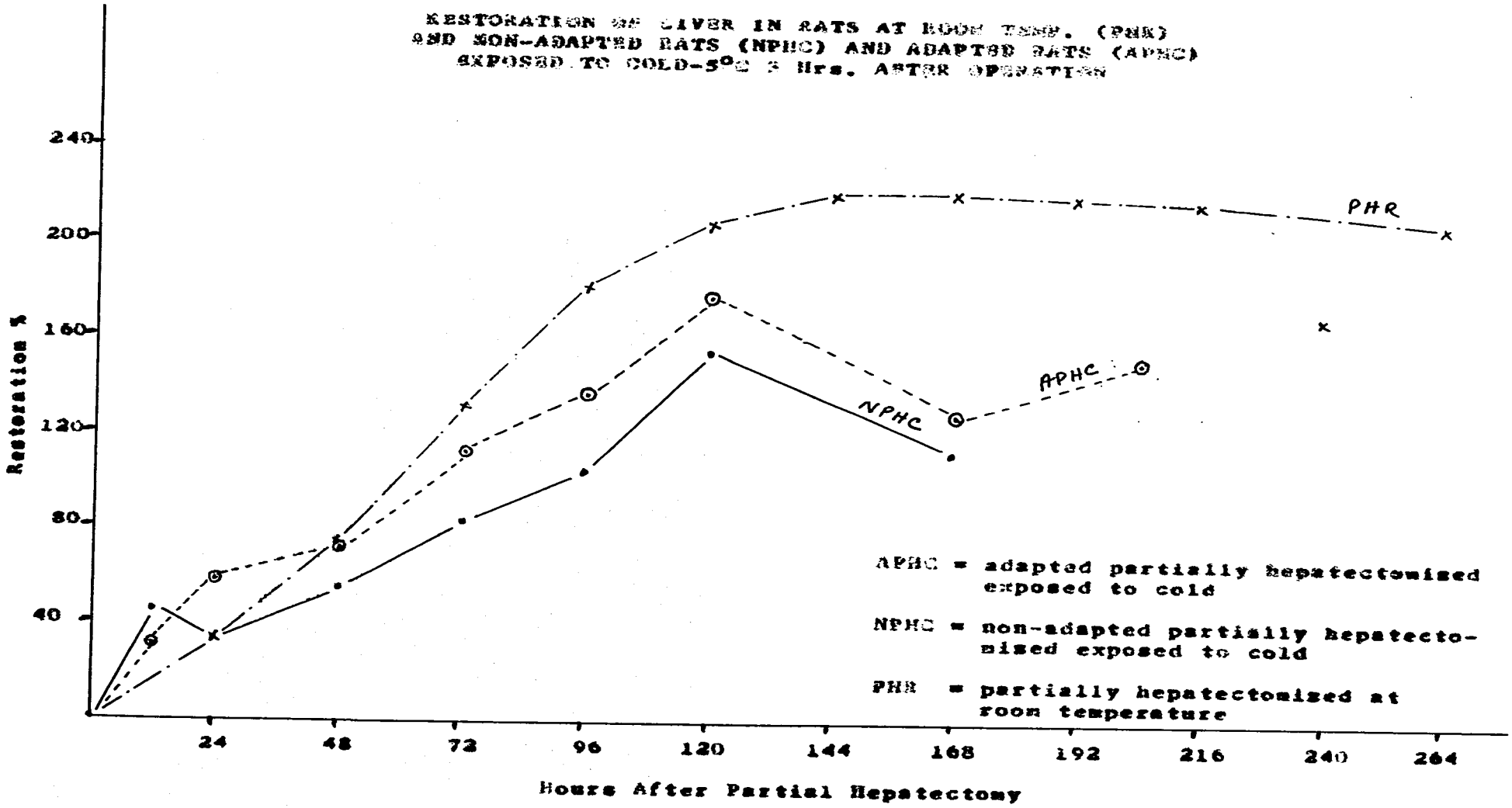

RESTORATION OF LIVER IN RATS AT ROOM TEMP. (PHR)
AND NON-ADAPTED RATS (NPHC) AND ADAPTED RATS (APHC)
EXPOSED TO COLD-5°C 3 Hrs. AFTER OPERATION
Restoration %
240
200
160
120
80
40
PHR
APHC
NPHC
APHC = adapted partially hepatectomized exposed to cold
NPHC = non-adapted partially hepatectomized exposed to cold
PHR = partially hepatectomized at room temperature
24
48
72
96
120
144
168
192
216
240
264
Hours After Partial Hepatectomy

Fig. 14

## SUMMARY

1° At room temperature (20±2°C), the vitamin C content of the restoring liver of the rat over a period of 11 days exhibited a cyclic phenomenon: 24 hours after operation it dropped followed by a rise 48 hours after operation; approached the initial value after 72 hours; on the 24 hours later dropped again to the level found at 48 hours after operation; remained constant from 96th to 168th hour; started to increase after 192 hours and attained the initial level on the 240th hour after operation.

2° The vitamin C content of non-adapted partially hepatectomized rats exposed to cold (-5°C) 3 hours after operation showed the same general pattern as those at room temperature over a period of 6 days.

3° It was observed that the level of vitamin C of restoring liver was lower in rats exposed to cold than in their controls at room temperature during the first 3 days of cold exposure.

4° In the case of adapted partially hepatectomized rats exposed to cold 3 hours after operation, the vitamin C content of the restoring liver followed

the same pattern as the ones at room temperature and as those of non-adapted rats exposed to cold (-5°C).

5° The vitamin C content of the restoring liver of the adapted rats exposed to cold was (i) slightly higher than the one of the non-adapted rats kept in the same condition at all times during the restorative period, and (ii) lower than the level of vitamin C of the restoring liver of the rats kept at room temperature for the first 3 days after partial hepatectomy.

6° The maximum restoration of the liver of the rat in terms of restoration percent, calculated after Weiss' formula (13), was found to occur both at room temperature and in the cold room between the 2nd and 4th day after partial hepatectomy.

7° The adapted and non-adapted rats exposed to cold (-5°C) 3 hours after partial hepatectomy had a slower restoration of the liver than the ones at room temperature especially after 5 days of operation. This does not support Weiss' observation that cold did not affect liver restoration (13).

8° The adapted partially hepatectomized rats showed a faster restoration than the non-adapted rats exposed to cold (-5°C).

40 The resistance of the partially hepatectomized rats exposed to cold (-5°C), was observed to vary according to the time elapsed between partial hepatectomy and exposure to cold (-5°C). The partially hepatectomized rats exposed to cold (-5°C) 48 hours after operation showed a remarkable survival of 80% at the end of 14 days in the cold and in contrast to the other groups exposed to cold 4, 6, 12, 24, 72 hours after partial hepatectomy. The partially hepatectomized rats exposed to cold 3 hours after operation were much less resistant to cold than the sham operated (survival 80%); if the partially hepatectomized were exposed 6 hours after operation the difference in survival between partially hepatectomized and sham operated was less but still sham operated showed better performance; 24 hours after operation the partially hepatectomized began to show increased resistance as the sham operated ones; 48 hours after operation the partially hepatectomized rats were decidedly more resistant than the sham operated rats and 72 hours after operation their resistance seemed to decrease. In all cases the partially hepatectomized and sham operated rats were more resistant than the non-operated controls.

(3) There was no variations in $QO_2$ of the non-fasted partially hepatectomized rats whether its determination was made 24, 48 or 72 hours after operation at the temperature of 28-30°C.

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