CHAPTER VII

CYTOCHEMICAL STUDIES
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Cytochemistry has been employed as one of the important tools in detecting the cytochemical changes in the azocarmine induced carcinomatous hepatic cell of guinea-pig. Marked cytochemical changes have been observed in the liver cells of guinea-pig treated with the potent hepatocarcinogen-azocarmine. Studies were conducted to detect the changes in glycogen, deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and vitamin C in qualitative level, which revealed a considerable variation in the different sets of experiment as compared to the control.

1. STUDIES ON GLYCOGEN:

Liver converts the simple sugar resulting from the digestion of carbohydrates into the form of a polysaccharide—glycogen, which is then stored in it for future utilization. Glycogen, being a storage product in the liver influences the metabolic process of the liver and might be actively related with the carcinogenic transformation in the hepatic cells. This stored product of carbohydrate metabolism is the essence of energy, thus the regulatory mechanism may be influenced by the cell constituent.

CONTROL SET:

The appearance of intense reddish violet granules indicated the presence of glycogen in the liver of guinea-pig. The control material was marked by the uniformity of the staining pattern in almost all hepatic cells (Plate No. 40) except the inner spaces of the blood vessels. Glycogen particles were seen to be
distributed uniformly in the walls of the arteriole and venule as that in the other components of the liver. Blank spaces were marked by the side of arteriole and venules. The cytoplasm was full of glycogen particles which exhibited intense reddish violet coloration. Prominent hexagonal cell cytoplasm was packed with glycogen particles.

**CARCINOGEN TREATED SET**:

Administration of the hepatocarcinogen-azocarmine had a marked effect on the liver of guinea-pig whereby the occurrence and distribution of the glycogen particles had been markedly effected. The deposition of glycogen particles occurred in certain areas while some were found to be devoid of glycogen. Thus there was qualitative change in the pattern of distribution of glycogen as a result of treatment. In some cases glycogen accumulated in the walls of the arteriole and venule. The hexagonal structure of the hepatic cells was found to be distorted.

After seven days of treatment, glycogen appeared as patches and a number of vacuolar spaces were visible in the liver cells (Plate No. 41). The uniformity of distribution of glycogen was lost and the interior of the arterioles and venules were blank with marked loss of glycogen. The accumulation of glycogen particles was increased in the walls of the arterioles and venules. The periphery of the cells had lesser amount of glycogen depletion than the central zone.
Administration of carcinogen to the guinea-pig for a continuous period of fourteen days had marked effect on the distribution as well as in the amount of glycogen. The amount of glycogen deteriorated on the whole but the periphery of the cells had a greater deposition than the central part of the liver cells. The blood vessels particularly its walls had a higher quantity of glycogen deposition in comparison to the other areas of the liver which was a marked feature of the 14 days treated liver of guinea-pig.

Invasion of the surrounding cells of the blood vessels and the deposition of the glycogen particles in it was marked after twentyone days of treatment. The walls of the blood vessels were filled up with glycogen particles while it was poorly deposited in the surrounding areas (Plate No. 42). Distinct vacuolar spaces were observed in which there was no glycogen which indicated a rapid glycogenolysis from existing glycogen vacuoles.

Administration of carcinogen to the guinea-pigs over a period of 28 days revealed a marked loss of glycogen indicating progressive glycogenolysis. Further, the distribution of glycogen particles was not uniform throughout the liver cells and also found to be very meagre.

The guinea-pig liver after 35 days of carcinogen treatment showed further deterioration in the pattern of deposition and distribution of glycogen. In the walls of the blood vessels and the invading areas of the liver sections some amount of glycogen was visible while the rest of the liver indicated
PLATE No. 40: Microphotograph of liver showing deposition of glycogen particles.

PLATE No. 41: Microphotograph showing the uneven distribution of glycogen particles in the liver after the administration of carcinogen.

PLATE No. 42: Microphotograph showing the accumulation of glycogen particles in the walls of the blood vessels and the invading tissues by the side of the blood vessels.
comparatively very poor glycogen reaction.

Continuous administration of azo-dye further reduced the deposition and occurrence of glycogen particles along with the appearance of some empty vacuolar spaces. Even the walls of the blood vessels showed a decline in the occurrence and in the pattern of deposition of glycogen in comparison to that of the control.

Further decline in the content of glycogen was marked after 49 days of carcinogen treatment. Appearance of a number of empty glycogen vacuoles indicated further acceleration in the process of glycogenolysis, showing very weak PAS reaction throughout the liver cells.

Progressive decrease in the occurrence and deposition of glycogen was revealed by the cytochemical reaction after 56 days of treatment of the guinea-pig liver. Most of the cells in this set did not exhibit glycogen reaction except a few scattered cells (Plate No. 43). This suggested a progressive glycogenolysis which was due to the administration of the hepatocarcinogen resulting in an imbalance in normal glycogen metabolism in the liver cells.

**VITAMIN C TREATED SET:**

Administration of vitamin C to the carcinogen treated guinea-pigs had a marked revival effect in its glycogen content. Although the uniformity in the distribution of glycogen was not fully restored. There was a marked revival
in the occurrence of glycogen and droplets.

After seven days of treatment in the vitamin C treated set of the experiment, the quantity of glycogen was not increased. No change in the pattern of glycogen distribution was detected. Reddish violet particles were visible in certain patches, while some areas of the liver showed a very poor glycogen reaction.

By the fourteenth day of treatment, marked changes in the occurrence and pattern of distribution of the glycogen granules were observed. The walls of the blood vessels exhibited a strongly positive glycogen reaction, although vacuoles were also observed (Plate No. 44). The amount of glycogen deposition was somewhat more and increased than that of the 7th day treated liver of guinea-pig.

By the 21st day of treatment deposition of glycogen was more marked particularly by the side of the arterioles and venules. The amount of glycogen increased gradually in the hepatic cell. But the occurrence and distribution of glycogen was not uniform which indicated that the glycogen metabolism had not attained the control level.

By the 28th day of treatment in the third set of the experiment, the amount of glycogen was further enhanced. Number of reddish violet particles were observed particularly surrounding the arterioles and venules and the deposition of glycogen particles were found to be more marked in the hepatic cells with markedly positive PAS reaction.
PLATE No. 43: Microphotograph showing the loss of glycogen after 56 days of treatment of the carcinogen azocarmine.

PLATE No. 44: Microphotograph showing the appearance of vacuolar spaces and an uneven distribution of glycogen particles.

PLATE No. 45: Microphotograph showing the appearance of glycogen particles in the liver section particularly surrounding the arterioles and venules.
Administration of vitamin C to the carcinogen treated liver of guinea-pigs after 35 days showed a marked revival effect in its cellular glycogen content (Plate No. 45). Although the occurrence of glycogen granules enhanced to a great extent, the uniformity in their distribution was still lacking in different parts of the liver.

Continuation of treatment with vitamin C for a period of 42 days had a marked revival effect on the glycogen content as well as in its cellular distribution. The amount of glycogen was enhanced and the occurrence and distribution of glycogen particles were progressively advanced towards the control state (Plate No. 46).

By the 49th day of treatment of the revival experiment the quantity and distribution of glycogen progressed much towards the control. The vacuolated spaces as was observed in the second set of experiment was lacking and showed a deposition of glycogen in patches.

The deposition of glycogen in the liver sections towards the later part of the revival experiment was marked. It had a tendency to maintain its uniformity in distribution (Plate No. 47) as the glycogen particles were not concentrated in the walls of the arterioles and venules.

Thus, the enhanced glycogenolysis induced in the carcinogen treated set of guinea-pig may be attributed to the interference of the azo-dye with the carbohydrate metabolism of the liver. As glycogen is the storage product in the liver,
therefore it may not be improbable that the carcinogen acted upon the glycogen synthesizing enzyme systems, thereby the synthesis of glycogen might be blocked and the stored glycogen was utilised for the rapid development of the cancerous cell. Another probability which cannot be ruled out is that — as the tumour growing process requires a huge amount of energy for its metabolism therefore all the glycogen got utilised in the process of tumour formation and carcinogenic transformation in cells which was revealed by progressive loss of glycogen from the liver cells.

2. STUDIES ON DEOXYRIBONUCLEIC ACID:

That the deoxyribonucleic acid (DNA) plays an important role in the genesis and control of carcinogenic growth is well known. Ryser (1971) illustrated in detail, how DNA might be related with the carcinogenic agents transforming them into carcinomatous growth. The cytochemical studies also demonstrated a variation in the DNA content and in its distribution pattern during the process of induced carcinogenesis in liver.

CONTROL SET:

DNA in the liver cells of the control set of the experiment showed a uniform staining of reddish purple colouration when stained with feulgen fuchsin showing a moderately positive feulgen reaction (Plate No. 48). The spaces of the blood vessels were blank and no DNA positive reaction was observed.
PLATE No. 46: Microphotograph showing the deposition of glycogen particles in liver section after the administration of vitamin C.

PLATE No. 47: Microphotograph showing the tendency of the liver sections towards the normal in its glycogen content.
CARCINOGEN TREATED SET:

The sections of this set was marked by the loss of DNA intensity as compared to that of the control. The uniformity in the distribution of DNA was lost. Another feature was the accumulation of the DNA particles by the periphery of the blood vessels. Further treatment of the carcinogen induced further loss of DNA intensity in hepatic cells as indicated by the feulgen reaction.

The administration of carcinogen for a period of seven days seemed to interfere with the DNA metabolism of the liver. The deposition of DNA was reduced to that of the control. Further, the uniformity in staining was hampered as the walls of the blood vessels and their peripheral areas were heavily stained while the rests indicated weakly positive feulgen reaction.

Further loss in the intensity of DNA was recorded after the administration of azocarmine for a continuous period of fourteen days as was revealed by poor feulgen positive reaction (Plate No. 49). The uniformity in distribution of DNA particles was lost and an uneven distribution was marked.

Continuous treatment with the hepatocarcinogen-azocarmine for 21 days had a fluctuating effect on the DNA content of the liver. The amount of DNA as shown by the feulgen fuchsin staining indicated marked fluctuation. After 21 days of treatment it slightly increased in intensity to that of seven days treated and 14 days treated materials but it remained below the control level.
PLATE No. 48: Microphotograph showing the DNA particles in the liver section of the control animals.

PLATE No. 49: Microphotograph showing the reduction of DNA staining and deposition of DNA particles by the side of the blood vessels after fourteen days of carcinogen treatment.

PLATE No. 50: Microphotograph showing the staining of DNA after 35 days of carcinogen treatment.
A decrease in the DNA content was observed after 28 days of carcinogen treatment which revealed a continuous interference and reaction of the carcinogen with the DNA synthetic machinery of the cell. The vacuolar spaces remained blank.

There was a similarity in the intensity of DNA of 35 day treated material with that of 28 days treated one. Lesser amount of DNA was observed, particularly in the peripheral side of the blood vessels which showed heavy deposition in the case of 14 days treated liver. Although the amount of DNA was reduced, it showed gradual tendency towards uniform distribution (Plate No. 50).

Further loss of DNA was observed after 42 days of treatment with the hepatocarcinogen-azocarmine. The nuclei of the liver cells responded to the feulgen staining but the intensity of reaction was low in comparison to that of the control. It was slightly feulgen positive.

Continuous treatment with azo-dye for a period of 49 days showed a lesser quantity of DNA than that of the control and the distribution was not uniform. Vacuolar spaces were observed which showed negative feulgen reaction signifying the marked loss of DNA.

56 days treated guinea-pig liver showed a general tendency of loss of DNA alongwith an uneven distribution pattern. Small patches appeared which stained somewhat deeper than that of the surrounding areas (Plate No. 51).
Administration of vitamin C to the carcinomatous guinea-pigs showed revival effect which indicated the probable interference of vitamin C with the carcinogenic changes induced by the hepatocarcinogen. It showed a progressive increase in the DNA content of the liver cells as it indicated strongly DNA positive reaction.

The 7th day treated material showed a reduction in the amount of DNA which revealed a loss of DNA from the liver of the guinea-pigs (Plate No. 52). Along with this, the distribution of the DNA was not found to be uniform.

The 14th day treated material showed further increase in the DNA content of the liver. It increased in amount above that of the control. The distribution pattern of DNA in the liver section was not found to be uniform up to 14th day of treatment.

Continuous treatment with vitamin C enhanced the DNA content in the liver cells above that of the control. The distribution of DNA appeared to be uniform except the periphery of the arterioles and venules which exhibited deeper DNA reaction than that of the surrounding area.

By the 28th day of treatment of the revival experiment the intensity of DNA was found to be increased and the distribution of DNA was not uniform. DNA accumulated by the side of the blood vessels (Plate No. 53).
**PLATE No. 51**: Microphotograph showing the staining and distribution of DNA after 56 days of carcinogen administration.

**PLATE No. 52**: Microphotograph showing the staining and distribution of DNA after 7 days in the revival experiment.

**PLATE No. 53**: Microphotograph showing the staining and distribution of DNA after 23 days of treatment.
By the 35th day of treatment the DNA reaction showed a partial revival in its quantity. But the uniformity in distribution of DNA was lost. The vacuolated spaces which appeared in the cell, in carcinogen treated set of the experiment gradually disappeared.

By the 42nd day of treatment the DNA content of the liver was observed to be above that of the control although it remained at a more or less constant level with the 28th day and 35th day treated guinea-pigs. The uneven distribution of DNA which was seen in the former sets of treatment was becoming uniform.

Continuous treatment of vitamin C further induced a revival in the quantity and distribution of DNA. The distribution of DNA was found to be uniform and the liver cells indicated a moderately positive DNA reaction.

By the 56th day of treatment the amount of DNA was found to be very high as indicated by strongly positive feulgen reaction and the distribution of DNA also attained uniformity of pattern (Plate No. 54). Vacuolar spaces completely disappeared signifying an increase in the amount and distribution pattern of DNA.

The above findings suggested that the administration of the hepatocarcinogen-azocarmine interfered with the DNA metabolism in the liver and reduced the DNA content in the carcinogen treated set of the experiment. But the administration of vitamin C interfered with the carcinomatous changes
PLATE No. 54: Microphotograph showing the staining and uniform distribution of P:A after 56 days of the revival experiment.
as induced by the carcinogen and a revival in the DNA content was observed due to the healing effects of vitamin C.

3. **STUDIES ON RIBONUCLEIC ACID:**

Studies were carried out for the detection of ribonucleic acid in liver sections in the control set, carcinogen-treated set and the vitamin C treated set of guinea-pig. Ribonucleic acid plays an important role in the metabolic processes of the body as it plays the key role in protein synthesis.

**CONTROL SET:**

Methyl green pyronin reaction of RNA in the liver cells of guinea-pig showed fine pinkish violet particles in the nucleoli and cytoplasm of the control animals (Plate No. 55). DNA particles were observed as blue particles. There was a uniformity in the distribution of DNA and RNA in the liver as indicated by moderately positive reaction for both DNA and RNA.

**CARCINOGEN TREATED SET:**

Administration of carcinogen had a drastic effect on the RNA content in the liver of guinea-pig which was markedly reduced to that of the control set of the experiment. Probably the carcinogen interfered with the RNA metabolism directly or indirectly and the quantity was reduced, due to this interference of the synthetic process.

The treatment of guinea-pig with the hepatocarcinogen-azo-carmine after 7 days reduced the RNA content of the liver as was evident from the occurrence of lesser quantities of RNA.
particles in the liver cells. The distribution of RNA was also not found to be uniform throughout the liver.

Continuous treatment for fourteen days resulted in further loss of RNA and the loss of the uniformity in distribution (Plate No. 56). The appearance of some empty vacuolar spaces in the cell was observed indicating gross anomalies in cellular physiology along with the interference with RNA metabolism.

Further loss of RNA was observed in the liver sections of 21 days treated guinea-pig. Loss of uniformity in the distribution of RNA was a marked feature which gradually became marked with the continuation of carcinomatous development.

Following 23 days of carcinogen administration, the RNA content showed a decline as observed in case of the 21 days treated liver. However, the intensity of RNA was found to be markedly lower than the control.

After 35 days of treatment, the RNA content of the liver exhibited a slight recovery than that of the 28 days treated guinea-pigs. The distribution pattern of RNA particles was not uniform in the cell and vacuolar spaces were prominent.

With the enhancement of time of carcinogen administration the RNA content showed a gradual decline in the liver of the treated guinea-pigs. 42 days treated liver cells indicated an overall decline in RNA reaction.

Continuation of the administration of the hepatocarcinogen-azocarmine up to 49 days showed a slight increase in the amount of RNA to that of the earlier treated set although it was below
PLATE No. 55: Microphotograph showing the staining of RNA in the nucleoli and cytoplasm in the liver of the control set.

PLATE No. 56: Microphotograph showing the loss of RNA and breakdown in the uniformity in distribution in liver cells after 14 days of carcinogen treatment.

PLATE No. 57: Microphotograph showing the loss of RNA in the liver section of guinea-pig after 56 days of carcinogen administration.
that of the control level.

After 56 days of treatment the RNA content was found to be less intense than the control and the distribution pattern of RNA was also not uniform (Plate No. 57). The marked feature in the 56 days treated material was that the cytoplasmic RNA More or less disappeared showing poor RNA reaction.

**REVIVAL EXPERIMENT : (VITAMIN C TREATED)**

Administration of vitamin C to the carcinomatous guineapigs interfered with the RNA synthesizing machinery and a revival effect was observed from that of the carcinomatous transformation in cytochemical level. Vitamin C is expected to produce some anticarcinogenic effects whereby the RNA synthesis showed a tendency of revival in the cellular level.

By the seventh day of treatment with carcinogen the RNA content of the liver was found to be decreased to that of the control. Alongwith the loss of RNA particles, its distribution became uneven.

By the fourteenth day of treatment in the revival experiment the amount of RNA was found to increase but the uniform distribution of the RNA particles was not restored (Plate No. 53). RNA was found to be present both in the nucleolus and cytoplasm. Accumulation of RNA particles was found by the periphery of the blood vessels.

There was a general tendency of the liver to regain the loss of RNA after the administration of vitamin C in the revival set of experiment. Vacuolar spaces persisted as in the carcinogen
treated set showing an impairment of protein metabolism after 21 days of treatment.

Continuous treatment of vitamin C by the 28th day showed a revival effect on the RNA content of the liver which decreased in the revival experiment. The distribution of RNA was not uniform throughout the liver cells.

By the 35th day of the revival experiment the RNA particles in the liver cells of the treated guinea-pigs slightly decreased to that of the 28th day treated material, but it persisted above that of the control level. The distribution pattern was found to be uneven.

Further increase in the RNA content was observed after fortissondo day of treatment in the revival experiment. Vacuolated spaces without RNA particles were still present (Plate No. 59). The distribution of RNA particles was not uniform.

By the 49th day of treatment the RNA content was found to be above that of the control level but the presence of blank and uneven distribution of RNA was still persistent.

By the 56th day of the revival experiment the RNA content of the liver of the treated guinea-pig was found to revive above that of the control level but the distribution was not found to be uniform, which indicated unequal RNA reaction.

Thus, the results revealed that the administration of the hepatocarcinogen-azocarmine interfered with the process of RNA synthesis and decreased the RNA content below the control level.
PLATE No. 58: Microphotograph showing the RNA staining in 14 days treated guinea-pig liver of the revival experiment.

PLATE No. 59: Microphotograph showing the RNA staining in 42nd day treated guinea-pig liver of the revival experiment.

PLATE No. 60: Microphotograph showing the staining and distribution of RNA after 56 days of treatment in the third set of experiment.
After the administration of vitamin C, RNA synthesis indicated a marked revival which was probably due to the healing and anticarcinogenic activity of the vitamin administered for the expected revival of the pathological changes in the liver.

4. STUDIES ON VITAMIN C:

Vitamin C is highly essential for regenerative and healing properties of cells and tissues. The present experiment revealed that this vitamin brought about a revival effect. On the carcinomatous changes in cytochemical level in terms of glycogen, DNA and RNA and with restoration of their normal amount which indicated the revival effect of vitamin C in case of induced carcinogenic changes.

CONTROL SET:

Liver sections were stained and examined for the presence of vitamin C. Black granular precipitate was observed scattered in the liver cells (Plate No. 61). The staining reaction indicated moderate and uniform occurrence of vitamin C in the liver cells.

CARCINOGEN TREATED SET:

The administration of the potent hepatocarcinogen-azocarmine in guinea-pig induced a visible variation in the vitamin C content in the liver cells of treated guinea-pigs, at the first phase of the second set of experiment but towards the later part of the experiment it decreased markedly and persisted to a more or less constant value.
After seven days of treatment the vitamin C content of the treated liver decreased greatly than that of the control.

A fluctuation in the vitamin C content was observed after fourteen days of treatment. There was an enhancement of vitamin C content in the fourteen days treated guinea-pigs to that of the control.

By the 21st day of treatment with the hepatocarcinogen the vitamin C content of the liver was again depressed below that of the control level. The vacuolar spaces which were found to be present in other sets of experiment were not so marked.

Continuous treatment with the carcinogen-azocarmine up to 23 days further reduced the content of vitamin C in the liver sections of treated guinea-pig. It was characterised by a very low deposition of black granular particles of vitamin C.

After 35 days of treatment with the carcinogen-azocarmine the vitamin C content was enhanced above that of the control level. This fluctuation of vitamin C content was particularly marked from fourteen days to 35 days of treatment in the second of the experiment.

There was a decrease in the stainability of vitamin C in the liver sections of guinea-pigs treated with carcinogen for a period of 42 days (Plate No. 62). The walls of the arterioles and venules also took the same uniform staining.

After 49 days of treatment there was further loss of vitamin C in the liver sections of the treated guinea-pig. However it remained in a level more or less equal to that of the 49th day of treatment.
PLATE No. 61: Microphotograph showing the staining of vitamin C in the liver cells of the control set of the experiment.

PLATE No. 62: Microphotograph showing the stainability of vitamin C after 42 days of carcinogen treatment.

PLATE No. 63: Microphotograph showing the stainability of vitamin C after 56 days of carcinogen treatment.
56 days treated liver cells showed a more or less similar precipitation of vitamin C with that of the 42 and 56 days treated liver. But it persisted below that of the control level (Plate No. 63).

**REVIVAL EXPERIMENTAL SET (VITAMIN C TREATED)**

The revival experiment was performed with the administration of vitamin C to the carcinogen treated guinea-pigs whereby the carcinomatous changes undergone by the liver in the second set of the experiment indicated a marked revival.

There was an enhancement in the quantity of vitamin C to that of the control in the seventh day treated material. The cells were full of black precipitates which revealed the presence of the vitamin in abundance.

By the fourteenth day of treatment in the revival experiment the stainability of the vitamin further increased showing an enhanced amount of the vitamin deposition in cells.

By the 21st day of treatment in the revival experiment the black granular precipitates of the liver cells decreased to that of the fourteenth day treated guinea-pigs but it was above that of the control, which definitely indicated an increase in vitamin C content.

By the 28th day of treatment there was a higher deposition of the black granular precipitate of the vitamin which revealed an increase in the accumulation of vitamin C in the cells.
By the 35th day of treatment the liver cells showed a slight depression in the vitamin C content than that of the 28th day treated material, but it persisted above that of the control.

A more or less constant deposition of vitamin C was observed in the 42nd day treated material with that of the 35th day treated material.

By the 49th day of treatment, the liver cells showed the presence of higher deposition of vitamin C than that of the control. The uniformity of distribution was not observed due to higher accumulation of vitamin C.

The stainability of the liver sections was much higher than that of the control in the 56th day treated material of the third set of the experiment. It was found to be maintained at an elevated level during the later part of the revival experiment.

The administration of the hepatocarcinogen-azocarmine interfered with vitamin C of the liver cells and its prevalence was lowered which may ultimately lead in the development of the lesions and tumours. But the administration of excessive quantity of vitamin C interfered with the carcinogenic changes and partial revival was obtained after vitamin C treatment.

6 DISCUSSION:

Bannasch (1968) described the cytochemical changes in the liver produced by N-nitrosomorpholine. According to him - first a reduction or loss of glycogen and second, a disaggregation
of the basophilic bodies typical of normal ergastoplasm, resulting in a diffuse cytoplasmic basophilia. Both the cytoplasmic changes are intimately related to each other and always occur together. The diffuse cytoplasmic basophilia may be more or less pronounced. In some cells the cytoplasm even shows a distinct eosinophilia and a fine granulation. The glycogen free cells are mainly found in the centre of the lobule and in small rays leading to the periportal regions. If a 6 mg. N-nitroso-morpholine (NNM) solution is given continuously throughout the experiment only rarely glycogen free diffuse basophilic liver epithelia was found in the vicinity of the central vein. He further reported that if a 20 mg. NNM solution was used, glycogen depletion and the concomitant cytoplasmic changes during carcinogen administration spread from the third to the second and in some cases even to the first zone of the functional liver acinus. As long as the administration of 20 mg. NNM solution continued it favoured cytoplasmic changes of the acinus central type over the acinusperipheral type; thus one could at first observe a marked depletion of glycogen in the liver parenchyma. In small parenchymal zones however, one could often witness a very pronounced glycogen storage especially in peripheral areas of the lobule. According to Dannasch and Muller (1964): NNM induced hepatomas always developed from parenchymal zones of enhanced glycogen storage. Cells of the peripheral zone showed the cytotoxic pattern more markedly and thus may be regarded as the immediate precursors of tumour cells. The beginning of the pathological growth is morphologically characterized by well defined alterations in the cytoplasm.
Along with the gradual transformation of a precancerous storage cell into a tumour cell the accumulated glycogen is gradually reduced until no more glycogen can be histochemically revealed in the cytoplasm. At the same time the net increase of the cytoplasmic basophilia (chromatogenes) was remarkable. The deplation of glycogen in the cancerous tissues may be due to the rapid utilization of energy by the growing cells. The findings on the lowered glycogen content of cancerous cells was also reported by Goranson et al. (1954), Orr and Price (1948), Orr et al. (1948), Graffi and Haberkerl (1953); Spain and Griffin (1957); etc. Hadjiolov and Dancheva (1958) reported that the failure of a hepatoma to store glycogen was due in part to the low activity of its phosphorylase due to partial inhibition of enzymatic activity.

The loss of nucleic acid in the liver cells in case of the present experiment may be due to the intervention of the carcinogen either directly or through the alteration of the enzymatic system. The inhibition of DNA is mainly due to the interaction with the carcinogen while the inhibition of RNA is due to the secondary effect. A 16.5 decrease of Feulgen DNA content in mice was noted in preleukemic and leukemic stages (Pereverzev et al. 1976). Godoy et al. (1976) studied the effects of prolonged feeding with aflatoxin B1 on adult rat liver and reported a loss of nucleic acid synthesis. Greenstein (1954) reported that whether the liver tumour was induced by an azo-dye or by aminofluorene, it ends up by being lower in protein, RNA and riboflavin per cell than is the normal liver.
Role of vitamin C as healing and anticytotoxic factor has been recognized from a long time. Cytologically vitamin C deficiency causes a reduction in cytoplasm, indistinct cell walls and loss of hyaluronic acid, the main intercellular cement (Baker, 1968). Vitamin C is necessary for the protection of intercellular substances having collagen, as an antioxidant it protects hydrogen electron carriers.

Normal cells form an integrated and interdependent community within the living organisms, and their growth is controlled by exogenous and endogenous factors in such a way that they are maintained in a state of dynamic equilibrium with other cell communities. It would therefore be expected that, alternations in any one, or a number of such exogenous and endogenous factors might affect the growth and maintenance of any one, or a number of the normal cell communities. Thus the deficiency of vitamin C results in a loss of control in the intercellular cementing matrix.

In mammals able to form L-ascorbic acid, the enzymes concerned with its synthesis are present in the liver microsomes whereas in chick and pigeon they are localised in the kidney microsomes. On the other hand, the livers of mammals unable to synthesize L-ascorbic acid have been shown to contain no L-gulono oxidase, the enzymes responsible for the conversion of L-gulonolactone to the corresponding 2-keto compound (Chatterjee, et al. 1961). There is evidence that L-ascorbic acid is interrelated metabolically with folic acid. In vitamin C deficient monkeys with megaloblastosis in the bone marrow,
markedly lowered levels of hepatic folic acid were found in comparison to that of the control (Kay et al. 1952). Laker (1957) reported that ascorbic acid played an important role in the metabolism of several amino acids. Thus, it is evident from the present experimental findings that the administration of vitamin C had definitely promoted a healing and revival effect in the cytochemical level in case of glycogen, DNA, RNA and ascorbic acid content of the liver cells showing a definite improvement on the intensity of DNA, RNA, PAS and vitamin C reaction.